PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 11 JANUARY, 1944 NUMBER 1

The effect of sludge digestion, drying and supplemental treatment on eggs of *Ascaris lumbricoides*.[1] ELOISE B. CRAM and DONALD O. HICKS, National Institute of Health.

There have been published elsewhere (1943, Sewage Works Jour., 15 (6): 1113–1138) the general results of tests of the survival of *Ascaris* and hookworm eggs and *Endamoeba histolytica* cysts subjected to various sewage treatment processes, with special emphasis on treatment of the effluent. However, it seems desirable to present in more detail the findings relative to the effect on *Ascaris* eggs of sludge digestion and drying. This phase of the problem is of special importance because the eggs of this parasite appear to have a greater resistance to external conditions than do other eggs or cysts of intestinal parasites of man. Moreover, in examinations made in 1941 (Wright, Cram and Nolan, 1942, Sewage Works Jour. **14**(6): 1274–1280) viable *Ascaris* eggs were found with relative frequency in sewage sludge from Army camps located in the southern United States. The increasing use of sludge as fertilizer in this country gives practical significance to the question concerning the fate of eggs of this parasite.

METHODS

For the present study, eggs of *A. lumbricoides* originating from man and swine were introduced into sewage taken from a municipal main in Cincinnati, Ohio; the sewage was then seeded with sludge obtained from a small Imhoff tank, usually in the proportion of 4 parts settled sewage to 1 part ripe sludge. Four- or eight-liter serum bottles were used as containers for the sludge in most instances. However, for 3 larger lots of sludge a miniature digestion tank with a floating cover was employed. To 10 parts of ripe sludge was added *daily* for 30 to 60 days 1 part of fresh settled sewage giving a yield of 60 to 72 liters of sludge at the end of the digestive process. The mixtures containing the *Ascaris* eggs were held usually for 2 to 6 months for observations as to the effect of digestion. After this time the sludge was poured for drying into wooden frames containing basic layers of gravel and sand of varying depths.[2]

To evaluate the influence of temperature, different lots of sludge were held at two constant temperatures, 68° and 86° F. (20° and 30° C.), respectively, as well as at varying temperatures of the laboratory, a greenhouse and outdoors on a porch roof. Ten- to 20-gram samples were drawn from the bottom of the experimentally inoculated bottle or tank, were plated, and were then held at 86° F. for observation for 14 to 30 days; samples of drying sludge were handled similarly. Viability of *Ascaris* eggs was judged by the development of active embryos. For computing the percentage of eggs still viable with lengthening of the period of digestion or

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the National Institute of Health.

[2] The writers are indebted to Mr. S. R. Weibel for help in designing and constructing the equipment and to Mr. O. R. Placak and Mr. W. A. Moore for analyses of the sludge at various stages. Other members of the Stream Pollution Investigations Station also cooperated generously.

drying, the results from simple washing and sedimentation were no more consistent than those obtained by flotation with zinc sulfate solution of a specific gravity 1.250. In fact the difficulty of detecting eggs by the former method was so great that its use was discontinued. The results reported are therefore based on the flotation technique.

SLUDGE DIGESTION

Of 18 lots of digesting sludge, 3 were inoculated with eggs of *Ascaris* of man and the remainder with eggs of this parasite from swine. For the most part there was no development of the eggs in the sludge. However, a small percentage was brought to the surface in the scum, probably with rising gas bubbles, and in such cases development of embryos proceeded in a normal manner.

The eggs were very resistant to the digestive process. Estimates of percentage viability of individual samples from the same lot and from different lots of sludge varied considerably; they could therefore be considered only as an approximation but from a large series of counts it was possible to follow the general level of persistence of viability. Averaging counts made on all lots held at various tempera-

TABLE 1.—*Effect on Ascaris eggs of sludge digestion at varying temperatures in a greenhouse*

	High temperatures °(F.)					Low Temperatures °(F.)			
Digestion time in days	40–50	50–60	60–70	70–80	80–90	19	30	40	51
No. days 80° or over	47	56	66	75	81	7	7	7	7
No. days 60° or below	3	3	3	7	15	19	30	40	51
Maximum temperature	115°	115°	115°	115°	115°	92°	92°	92°	92°
Minimum temperature	56°	56°	56°	52°	40°	36°	34°	34°	34°
Per cent viability of eggs after various time periods									
Lot No.									
15	100	88	94	96	97				
16	97	98	77	87	83				
17		76	24	52					
13			87	75	68				
26						89	51	19	8

tures, approximately 70 per cent of the eggs were viable during the first 3 months, the period of most active digestion; thereafter, in sludge which by that time was usually well ripened, there was a sharp drop in *Ascaris* viability, but at the end of 6 months an average of 10 per cent of the eggs were still capable of developing active embryos.

The percentage survival of eggs from *Ascaris* of man exceeded this average in one lot digested at 68° F.; in this lot there was an average viability of 38 per cent after 120 to 140 days digestion. Two lots were less resistant at 86° F.; in one lot an average of 9 per cent of the eggs were viable in the period between 120 to 140 days, with a drop to an average of 5 per cent during the next 20 days, and an absence of viability at 160 days and thereafter. In the other lot an average viability of 42 per cent after 60 to 80 days dropped to 4 per cent in the period between 120 and 140 days, and no viable eggs were found after 143 days of digestion.

At 68° F. *Ascaris* eggs of swine origin were still viable after 321 and 371 days, respectively, in two lots. The persistence of high levels of viability during digestion at high summer temperatures in the greenhouse is shown in table 1 for 4 lots of sludge as compared with a more rapid loss of viability in one lot digested at lower winter temperatures in the same location.

EFFECT OF REPEATED SLUDGE DIGESTION

Two tests were conducted to determine the effect of repeated, as compared with a single, sludge digestion. Eggs were recovered from sludge after digestion and were again introduced into a 4 to 1 mixture of fresh settled sewage and ripe sludge. In the one case primary digestion at 86° F. for 53 days was followed by secondary digestion at the same temperature. Under the latter conditions approximately 19 per cent of the eggs were still viable after 87 days of secondary digestion but no viable eggs were found after 97 days of secondary digestion or after a total of 150 days covered by both primary and secondary digestion. In the other lot primary digestion for 86 days at high summer temperatures in the greenhouse (Lot 17, Table 1) was followed by secondary digestion at laboratory room temperature. In this lot viability persisted longer; approximately 30 per cent of the eggs were viable after 180 days of secondary digestion or a total of 266 days covered by both digestion periods. Judging from these two lots, repetition of the digestive process was not notably more deleterious to *Ascaris* eggs than was their continued presence in the original sludge.

AEROBIC FOLLOWED BY ANAEROBIC DIGESTION

Under the aerobic conditions found in activated sludge, active embryos developed in *Ascaris* eggs which were unsegmented at the time they were introduced in sewage. To compare the resistance of embryonated with non-embryonated eggs as used in the previous experiments, two lots of activated sludge containing embryonated *Ascaris* eggs were subsequently held as digesting sludge. After 65 days of aeration one lot was mixed in the ratio of 2 parts activated sludge, 1 part ripe Imhoff sludge, and 2 parts of freshly settled sewage and was held for anaerobic digestion at 68° F. Embryos were still alive 108 days later. This lot was subsequently dried (see below). In the second lot after 29 days aeration, 1 part activated sludge and 1 part fresh settled sewage were digested at 68° F.; active embryos were found for a period of 112 days of anaerobic digestion but on subsequent examinations no activity could be seen. It would appear therefore that embryonated eggs survive anaerobic digestion for periods in excess of those usually allowed for sludge digestion although the limited evidence available suggests that they are not as resistant as are non-embryonated eggs.

SLUDGE DRYING

Six lots of digested sludge, containing *Ascaris* eggs, were dried in the greenhouse. The period of digestion, the chemical analysis of the sludge when poured, details concerning the drying bed, and the results of drying are shown in table 2. The first 3 lots (Nos. 7, 2 and 4) were poured into a total of 8 compartments to evaluate differences in depth of gravel, sand,[3] and sludge. No *Ascaris* eggs appeared in the effluent from compartments having a 6-inch sand layer, whereas considerable numbers passed a 2-inch and small numbers passed a 4-inch layer. These cakes were dried during the summer months when the temperature in the greenhouse was frquently above 100° F. with a maximum of 115° F. Eggs in the upper layers embryonated as aerobic conditions obtained. As noted in table 2, the ova were extremely resistant to drying; 6 of the 8 cakes were removed after 18 to 81 days drying, while viability was still present, and the moisture content of these cakes was found to range from 5.8 to 11.5 per cent. Two cakes held until the ova were non-viable proved to have a moisture content of 3.3 and 4.2 per cent after 79 and 78 days of respective drying.

[3] Sand analysis: Effective size 0.53; uniformity coefficient 1.47.

TABLE 2.—*Effect on Ascaris eggs of sludge drying at varying temperatures in a greenhouse*

Lot No.	Digestion		Sludge, per cent		Bed dimensions			Days drying	Viability (per cent if known)	Drying temperature °(F.)				Per cent moisture
	Days	Temp.	Solids	Ash	Gravel	Sand	Sludge			Max.	Min.	Days 70 or over	Days 32 or below	
7	53	86° F.	1.8	54.1	12″	2″	6″	18	+	112	60	18	0	6.8
					9	2	9	51	+	115	56	51	0	6.8
					6	2	12	79	−	115	52	79	0	3.3
2	88	86° F.	3.7	37.7	8	6	6	35	+	115	60	35	0	10.6
					5	6	9	51	+	115	56	51	0	11.5
					2	6	12	81	+	115	56	81	0	5.8
4	133	86° F.	7.9	25.8	8	6	6	78	+	112	36	67	0	6.9
					12	4	4	78	−	112	36	67	0	4.2
9	139	R.T.	3.7	58.4	13	3	4	104	25	92	1	17	38	7.9
10	113	R.T.	2.6	56.8	2	6	12	118	6	92	0.5	22	41	56.7
26	71	G.H.	11.9	52.6	4	6	9	107	6	78	0.5	1	42	51.2

The remaining 3 lots of sludge dried in the greenhouse were held for longer periods extending into the winter. During this time the temperature fell below freezing more than one-third of the time. Nevertheless, 25 per cent of the eggs were viable after 104 days in one cake with final moisture content 7.9 per cent and 6 per cent of the eggs in the other two cakes were viable after 118 and 107 days, with final moisture content somewhat above 50 per cent.

For comparison with indoor drying where the drop in moisture content was progressive but where direct sunlight was absent, 13 cakes of drying sludge were exposed to the elements on a porch roof. The moisture content varied greatly with the weather; when determinations were made in comparatively dry periods it ranged

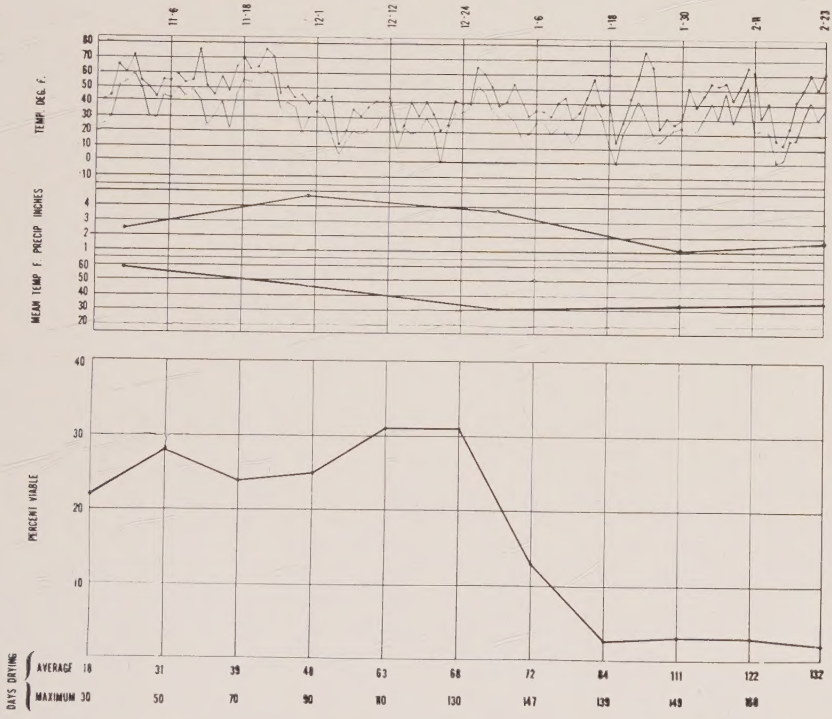

FIG. 1. Outdoor drying of sludge. From top to bottom: Temperature (maximum and minimum, degrees F.); precipitation (inches); mean temperature (F.); per cent viability correlated with days drying (average and maximum).

from 30 to 58 per cent. In only 3 of the 13 cakes was there failure to find viable ova at the end of the observation period. In one of these the eggs had become embryonated prior to digestion as a result of initial activated sludge treatment; in this cake active embryos were found at the end of 36 days drying but no motility was discernible after 42 days. Of the other two cakes with final loss of viability, one had been exposed for 61 days, on 46 of which the temperature was above 70° F. with only 1 day of freezing, whereas the other cake had been exposed for 73 days, on only 4 of which did the temperature reach 70° F. with 47 days of freezing temperatures. Figure 1 presents the meteorological data during the last 4 months of roof drying as well as the average of all counts conducted to determine viability of *Ascaris* ova in the sludge.

Information concerning the digestion and drying periods and the findings as regards persistence of viability of *Ascaris* eggs are presented in table 3. In sum-

TABLE 3.—*Viability of Ascaris eggs after digestion and drying of sludge under various conditions*

Temperatue of digestion	Length of digestion, days	Type of drying	Viability of *Ascaris* eggs	Number days drying	Moisture content of sludge, per cent
68° F.	139	At room temp.	+ −	29 35	9.9 at 19 days Less than 5
	108[a]	Outside	+ −	36 42	
86° F.	133	Greenhouse	+ −	78 78	6.9 4.2
	41 to 95	Greenhouse	+[b] −	18 to 81 79	5.8 to 11.5 3.3
		Outside	+[c] + [d] − [d] + [d] − [d]	42 to 171 42 61 63 73	 58.4
Greenhouse	71	Greenhouse	+	107	
		Room temp.	+ −	47 56	27.0
		Outside	+	104	
Room temp.	113 to 139	Greenhouse	+ +	104 118	7.9 56.7
		Outside	+	37	

[a] After 65 days activated sludge treatment.
[b] Five cakes.
[c] Four cakes.
[d] Same cake.

marizing these data, it would appear that *Ascaris* eggs cannot survive reduction of the moisture content below 5 per cent. Except for that factor, the cases where there was apparently loss of viability do not fall into any certain categories as regards temperature and extent either of digestion or drying. Failure to find viable eggs in one or several samples of sludge was not proof of complete destruction of viability as was evidenced by subsequent positive findings in some lots of sludge; more extensive sampling of some cakes here reported as negative might therefore have shown them to be positive. However, irrespective of this possibility, it appears clear from the preponderance of positive findings that *Ascaris* eggs may survive long periods of digestion and drying of sludge.

EFFECT OF STORAGE ON DRIED PULVERIZED SLUDGE

A sludge cake (Digestion lot No. 13, Table 1) dried for 55 days on the roof and with a 30.7 per cent moisture content showed on examination approximately 63 per cent of the *Ascaris* eggs viable; the sludge was pulverized and part was stored at 68° F. in a tightly covered glass dish. Forty-four days later there was no evidence that the viability of the *Ascaris* eggs had been reduced. A count at that time showed 77 per cent viable; the discrepancy between this and the original 63 per cent viability is illustrative of variations sometimes found in different samples from the same lot of sludge, as noted previously. The sludge was then left in a loosely covered dish which allowed slow drying; 71 days later the moisture content was 4.5 per cent and no viable eggs could be found.

EFFECT OF HEATING OF SLUDGE

Part of the same lot of pulverized sludge referred to above was utilized for tests to determine whether moderate heating for short periods would destroy the

TABLE 4.—*Effect of heat on viability of Ascaris egge in sludge*

	50° C. (122° F.)				Control	103° C. (217° F.)	
Minutes heated	3	5	10	20	Not heated	1	3
Moisture content (per cent)	29.2	28.9	28.3	26.2	30.6	28.8	26.7
Loss of moisture (per cent)	1.5	1.7	2.4	4.5	–	1.8	3.9
Viability	+	+	+	+	+	+	–
Per cent viable	33	36	26	29	63	41	0
Heat effectivity (per cent)	47	43	59	54	.....	35	100

viability of *Ascaris* eggs. Samples weighing approximately 3 grams were spread in a thin layer, approximately $\frac{1}{16}$ inch thick, in a Petri dish of $3\frac{1}{2}$ inch diameter. Two series of tests were run, one at 50° C. (122° F.), the other at 103° C. (217° F.). Duplicate samples were heated in each case and moisture determinations subsequently made. Water was added promptly to those samples which were to be examined for remaining viability. To allow for embryonating the cultures were held at 86° F. for 12 days, at the end of which time a zinc sulfate flotation of the entire sample was made. A nonheated control sample of the pulverized sludge showed a 63 per cent viability; this was used as a basis for computing the heat effectivity in each test. The results are shown in table 4. Approximately 29 per cent of the *Ascaris* eggs were still viable after heating at 50° C. for 20 minutes, with a loss of approximately 4 per cent moisture; the computed heat effectivity was therefore only slightly over 50 per cent. On the other hand, heating for 3

minutes at 103° C., with a resultant 4 per cent drop in moisture content, destroyed 100 per cent of the *Ascaris* eggs. The shell of the eggs had lost its transparency and the protoplasm appeared globulated.

METHYL BROMIDE FUMIGATION OF ASCARIS EGGS IN SLUDGE AND SAND

Four samples, 3 of sludge and 1 of sand, all containing *Ascaris* eggs were used for tests with methyl bromide. The eggs in the sludge had previously undergone sludge digestion and drying. Those in the sand had been introduced 24 days previously in fresh sewage in connection with tests of intermittent sand filtration. After filtration the sand had been allowed to stand until eggs in the upper portions became embryonated; the material was then used for the present test. A plot of soil 8 feet square was enclosed by a wooden frame which extended 10 inches below and 2 inches above the surface. The samples were placed in the soil so that the top was level with the surface and the plot was covered with kraft paper which had been treated with a compound[4] which rendered it water-proof and gas-tight. The paper was stretched tightly over the frame and carried over the sides so that it too extended 10 inches below the surface of the soil, which was tightly packed against it. Through an opening in the paper a one-pound can of methyl bromide was inserted and the can pierced so as to allow escape of the gas, after which the

TABLE 5.—*Effect of methyl-bromide fumigation on Ascaris eggs in sludge and sand*

Kind of sample	Size of sample	Per cent moisture	Egg stage at exposure	Per cent embryonation of eggs after exposure	Per cent embryonation of eggs in control
Sludge #1	5″ diam. 1″ depth	44.5	90% one-cell 10% two-cell	8	38
" 1a	3″ diam. 1½″ depth	35.3	93% one-cell 7% two-cell	4	52
" 2	6″ × 6″ × 1″ depth	31.9	one-cell	0	36
Sand	10″ × 20″ × 2″ depth	45.0	Active embryos	5 to 45 (Avg. 24)	32

paper cover was quickly sealed. The soil temperature at time of fumigation was 61° F., the sludge temperature 68° F., and the range of air temperature during the following 24 hours 61° to 68° F.

Upon removal of the samples after 24 hours, eggs were examined for signs of disintegration or for death of embryos. Samples were plated and incubated for 14 days at 86° F. and examinations made on 12 of these 14 days. The results are shown in table 5. In sludge Numbers 1 and 1a, 10 and 7 per cent of the eggs, respectively, had segmented before the start of the experiment; the remainder were undeveloped. In control cultures made from untreated samples of these two lots of sludge development proceeded to embryonation in 38 and 52 per cent of the eggs, respectively. On the other hand, subsequent to fumigation with methyl bromide only 8 and 4 per cent, respectively, of the eggs continued development to embryonation in these two lots of sludge. The percentages of *Ascaris* eggs which survived fumigation therefore corresponded fairly closely to the original percentages of eggs in which development had already been initiated at the time they were exposed to the gas. There was little evidence of disintegration of protoplasm in

[4] Soldine. From Soldine Corporation, Evanston, Ill.

the gassed eggs; those which failed to develop after fumigation appeared for the most part as apparently normal 1-cell eggs and, in small numbers, as eggs in which early development had been suspended.

In sludge lot number 2, in which all *Ascaris* eggs were in the undeveloped 1-cell stage prior to the test, eggs in the gassed sample failed to show any evidence of viability during the ensuing incubation period of 14 days, whereas in the ungassed control 36 per cent of the eggs became embryonated. On the other hand, the effect of methyl bromide fumigation on embryonated eggs in sand appeared to be relatively slight; in 12 counts made during the 14-day incubation period following treatment, from 5 to 45 per cent, with an average of 24 per cent, of the eggs contained active embryos, as compared with active embryos in 32 per cent of the eggs in the ungassed control sample.

From this experiment, therefore, the evidence indicated that methyl bromide was lethal to *Ascaris* eggs before the start of their development but that the gas had little effect on the continued viability of developing eggs or on eggs with active embryos. The results differed from those of Andrews, Taylor and Swanson (1943, Proc. Helminth. Soc. Wash. **10**(1): 4–6). They found no evidence of viability after 20 hours of methyl bromide fumigation of *Ascaris* eggs in various stages of development. In their experiments the eggs were contained in charcoal cultures of swine feces placed on the surface and also buried 2, 6, and 12 inches below the surface of sandy loam soil.

SUMMARY

Eggs of *Ascaris lumbricoides* introduced with fresh settled sewage into digesting sludge survived for long periods. An average of 10 per cent was viable after 6 months in digesting sludge and even after a year some eggs were still capable of development. During digestion high summer temperatures appeared to have less effect than did low winter temperatures. Repeated sludge digestion was not notably more effective than was a single digestive process. Eggs previously embryonated in activated sludge appeared to be somewhat more susceptible to subsequent anaerobic digestion than were undeveloped eggs. *Ascaris* eggs survived drying to a point where the moisture content of the sludge reached 5.8 per cent but failed to survive when the moisture content reached a lower figure. No loss of viability was apparent during 44 days storage of pulverized sludge under conditions which prevented further drying. Heating of pulverized sludge to 103° C. (217° F.) for 3 minutes destroyed 100 per cent of the *Ascaris* eggs. Fumigation of eggs in sludge and in sand with methyl bromide for 24 hours destroyed those which had not started development but had little effect on partially or completely developed eggs.

Preliminary observations of the control of worm parasites in swine by the use of skim milk. L. A. Spindler, Harry E. Zimmerman, Jr., and C. H. Hill, U. S. Bureau of Animal Industry.

INTRODUCTION

It has long been recognized that a milk diet will often bring about marked changes in the bacterial flora of the intestine. Liefmann (1909, München Med. Wchnschr. **56**: 509–511) suggested that feeding sour milk might be an effective means of eliminating typhoid bacilli from carriers. Hegner (1923, Amer. Jour. Hyg. **3**(2): 180–200) reported that an exclusive milk diet is disadvantageous for the growth of such protozoans as *Giardia*, *Trichomonas*, and *Hexamitus* in rats. Porter (1933, Amer. Jour. Hyg. **22**(2): 467–474) found that a milk diet increased the susceptibility of rats to infection with *Nippostrongylus muris*. Foster (1936, Amer. Jour. Hyg. **24**(1): 109–128) found that 1 dog and 4 cats became "distinctly

more susceptible to hookworms when they had been fed for several weeks on a milk diet.'' So far as the writers are aware, the only references to the effect of a milk diet on intestinal helminths of livestock are those of Porter (1941, Jour. Parasit. 27(6) (Dec. Suppl.): 18–19), and Leese (1943, Vet. Jour. 99(10): 276). Porter concluded on the basis of numbers and sizes of worms found at necropsy that calves fed only skim milk were less susceptible to experimental infections of *Haemonchus contortus* than were control calves fed ''a normal diet.'' Leese reported that administration of cow's milk to colts infected with strongyles was highly beneficial even in the case of animals that ''were almost moribund (unable to rise without assistance) before treatment was applied.''

During the course of investigations on swine parasites at the field station of the Zoological Division, Beltsville Research Center, Agricultural Research Administration, brood sows and pigs of varying ages have been fed at times skim milk as a supplement to the regular maintenance ration of grain. The milk was fed whenever it could be obtained and in the quantities available. It was observed that the animals fed milk usually remained free of whipworms, nodular worms, and ascarids, even though opportunities for acquiring these parasites were often afforded through contact with soil that was unavoidably contaminated with infective stages of the worms named. It was observed also that ingestion of large quantities of milk by the animals was often followed by copious diarrhea which lasted for varying periods. In view of these facts an investigation was begun in December, 1942, to ascertain whether administration to infected hogs of skim milk in quantities large enough to produce purging would remove intestinal worms from these animals. The results of this investigation are briefly reported in this paper.

PROCEDURE

Twenty-six hogs of mixed breeds, ranging in age from approximately 4 months to 1 year, were used. These hogs harbored natural infections of one or more of the intestinal helminths commonly found in swine; the parasites were chiefly ascarids (*Ascaris suis*), nodular worms (*Oesophagostomum* spp.), and whipworms (*Trichuris suis*). To facilitate administration of the milk and the collection of feces, the animals were placed in individual pens having a smooth concrete floor and built-in concrete troughs. Preliminary fecal examinations were made to ascertain the kind of intestinal helminths present and whether any were being spontaneously expelled by the hosts. In the entire series of hogs involved in this investigation only 1 immature ascarid was so expelled.

All solid feed and water were then withheld for 3 to 5 days and fluid skim milk fed *ad libitum*. In the case of the pigs which drank milk readily, the milk diet was discontinued after 3 days; some pigs did not drink the milk readily at first, and in these cases the period that they were maintained on milk was extended to 4 or 5 days, depending upon the avidity with which it was consumed.

Fourteen of the pigs herein designated as group 1 were kept under close observation during the period they were maintained on milk. During the day the feces were collected as soon as possible after they were voided, and placed in water; feces passed during the night were collected the next morning. The 24-hour accumulations of feces were then screened to recover any intestinal helminths that might be present. At the close of the milk-feeding period the hogs were necropsied to ascertain (1) whether they still harbored intestinal helminths and (2) whether any pathological conditions were present that might be attributed to the milk diet. Data with regard to the number of worms passed by the individual animals are summarized in the accompanying table.

The remaining 12 animals herein designated as group 2 were fed skim milk for periods of 3 days in the manner described for group 1, but the feces were not

TABLE 1.—*Number of intestinal helminths expelled by hogs while maintained on a diet of skim milk*

Pig number	Time on milk diet	Whipworms expelled		Nodular worms expelled		Ascarids expelled	
	Days	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
1292	3	13	100.0	198	100.0	18	40.90
1303	3	114	90.47	82	100.0	16	94.11
1304	3	57	100.0	526	100.0	22	66.66
1305	3	63	100.0	28	100.0	10	8.92
1306	4	26	96.29[a]	30	90.90[a]	7	24.13
1307	3	98	100.0	182	99.45	5	13.51
1308	3	6	85.71	29	96.66[a]	28	50.90
507	3	Not infected		218	99.09[a]	0	0.0
1267	3	do		108	90.75	Not infected	
1321	3	2	100.0	154	100.0	27	67.50
1289	3	10	100.0	47	94.00	0	0.0
1275	3	25	100.0	183	100.0	51	75.00
1291	3	99	99.0[a]	450	100.0	Not infected	
1309	5	27	61.36	21	95.45	40	31.49
Total		540	94.40	2,256	99.03	224	36.68

[a] The worms not expelled were males.

collected and examined. These animals were not necropsied, but were returned to the regular grain ration to ascertain whether their appetite had in any way been impaired by the milk diet. Fecal examinations on alternate days were made on these animals during a period of 3 weeks after they were returned to a grain ration to ascertain whether any helminths capable of egg production remained. Data relative to this group of hogs are summarized in the text.

EXPERIMENTAL FINDINGS

An examination of the table shows that from 61.36 to 100 per cent of the whipworms, from 90.75 to 100 per cent of the nodular worms, and from 0 to 94.11 per cent of the ascarids were lost by the group 1 pigs during the period that they were maintained on the milk diet.

All the group 2 pigs harbored infections of whipworms and nodular worms but not ascarids at the time they were placed on the milk diet. The feces of all remained free of whipworm eggs, and the feces of 11 remained free of nodular worm eggs for a period of 3 weeks after the pigs were returned to a grain diet. In the case of the remaining animal, nodular worm eggs were found in a 20-gram sample of feces collected 2 weeks after completion of the milk treatment, indicating that a very small number of these worms was not removed. Three to six months later all animals harbored light infections of nodular worms as they had been maintained in contaminated pens and lots. This showed that an immunity to reinfection with this parasite did not exist following loss of worms during the time the pigs were maintained on the milk diet; no whipworm infections had been acquired.

DISCUSSION AND CONCLUSIONS

As can be seen from data presented in this report, 26 hogs were freed of the vast majority of the whipworms and nodular worms; in the case of 14 of these animals that were infected with ascarids smaller numbers of these parasites were removed. Removal of worms was accomplished by withholding all solid feed and water for a period of 3 to 5 days and feeding skim milk *ad libitum*. The skim milk produced a copious diarrhea which usually began within a few hours. In the case of pigs that drank the milk readily and thus became diarrheic within a few

hours the vast majority of the nodular worms were expelled during the first and second days. Usually during the latter portion of the second day the grain contents of the cecum were passed; the whipworms were then expelled during the remainder of that day and the following day; ascarids were expelled at irregular intervals. In the case of pigs that did not drink milk readily, purging was less complete and fewer worms were eliminated. It was found unnecessary to continue administration of milk after the fecal material became frothy as very few worms were expelled after that time; frothy feces usually appeared after the diarrhea had persisted about 3 days.

The liquid diet and the resulting diarrhea apparently produced no ill effects on the pigs since, with one exception, all the animals gained weight. In the case of the animals that were not necropsied (group 2), but were returned to a grain diet, no impairment of appetite was noted. In the case of 2 of the group 1 pigs a low-grade enteritis was observed at necropsy. Low-grade enteritis has from time to time been observed by the authors in pigs maintained on a grain diet; consequently, the condition in the animals in question is considered to be of doubtful significance.

The amounts of milk which the various animals consumed daily varied with the size and other factors but ranged from approximately 2 gallons for the smaller pigs to 7 or 8 for the larger animals.

The findings indicate that it may be possible to keep at a low level infections of nodular worms, whipworms, and ascarids in pigs by frequent administration of skim milk. Preliminary observations, as yet unpublished, showed that growing pigs maintained under conditions of constant exposure to infection remained relatively free of the parasites under discussion, when fed sufficient milk once each day to produce purging. These animals also made more rapid gains and remained in better health than comparable control pigs, which acquired heavy infections of nodular worms, whipworms, and ascarids.

Further observations on the pathogenicity of *Strongyloides ransomi* to swine.

L. A. Spindler, U. S. Bureau of Animal Industry.

Studies on the intestinal threadworm of pigs, *Strongyloides ransomi*, carried out at the Zoological Division Station, Beltsville Research Center, Agricultural Research Administration, have shown that natural as well as experimental infections may cause serious injury or death (Spindler, Hill, and Zimmerman, 1943, N. Amer. Vet. 24(8): 475–486). Additional observations on a brood sow and her pigs infected with *Strongyloides* indicate that this parasite is a potential menace to hogs as well as to pigs. These observations are briefly summarized in this paper.

Sow 1119, harboring a light infection of *Strongyloides*, farrowed on May 31, 1943, a first litter of 11 apparently healthy pigs; during the suckling period the sow and litter were maintained under conditions of scrupulous cleanliness (Spindler, 1942, Proc. Helminth. Soc. Wash. 9(1): 22–23). The *Strongyloides* infection harbored by the sow probably was the residuum of an experimental infection of approximately 1 year's duration and was so light at farrowing time that it could be demonstrated only by culture methods; no increase in numbers of eggs in the feces was observed during lactation.

During the first 3 weeks the young pigs remained healthy and grew rapidly; fecal examinations made at irregular intervals did not show the presence of any parasite eggs or coccidial oöcysts in the dejecta. Between the third and fifth weeks one pig after another became diarrheic and listless; labored breathing was noticeable; the diarrhea and labored breathing subsided after about 1 week. Salt flotation examinations made on feces of the pigs after the diarrhea subsided showed that all were infected with *Strongyloides*. The pigs remained listless, were inactive,

and failed to grow materially for a period of about 6 weeks; after that time they began to gain weight slowly.

On July 7 one of the pigs died suddenly. On post-mortem examination it was found to be emaciated and exceedingly rough in appearance. From the small intestine about 372 adult *Strongyloides* were recovered; no other intestinal helminths were found nor were coccidial oöcysts found in the intestinal contents. The brain, spinal cord, tongue and its extrinsic muscles, myocardium, the muscles of one front leg including the shoulder, and the muscles of one hind leg including the ham were each ground through a food chopper and examined separately for the presence of *Strongyloides* larvae; the technique has already been described (Spindler, 1937, Proc. Helminth. Soc. Wash. 4(2): 62–63). Live third-stage threadworm larvae were recovered from all the tissues examined except the brain and spinal cord. The weight of each tissue and the number of larvae recovered are as follows:

Myocardium, 24 grams, 17 larvae; tongue, 22 grams, 30 larvae; fore leg and shoulder, 43 grams, 32 larvae; hind leg and ham, 71 grams, 80 larvae.

As no other conditions were found that could be considered responsible for the ill health and death of this pig, it seemed reasonable to conclude that they were a result of the parasitic infection in the course of which the heart and skeletal musculature were extensively invaded by the larvae.

The remaining 10 pigs were weaned on the morning of July 28 and the sow was transferred to an outdoor lot which was partially shaded by trees. A few hours later she was observed to be lying down and apparently exhausted. Since the day was hot she was transferred to an indoor pen and kept cool by frequent spraying with cool water. Death occurred during the night.

At necropsy about 280 adult *Strongyloides* were recovered from the small intestine and 77 live third-stage larvae were recovered from a 100-gram sample of the finely-ground myocardium. No other helminth parasites, and no coccidial oöcysts were found; no other conditions that could be considered as responsible for death of the animal were observed.

At this station, during the past 10 years, sows of varying ages not harboring *Strongyloides* infections have regularly been transferred at weaning time, both in summer and in winter, to an outdoor lot. Many of these sows were in poor physical condition as a result of suckling large litters of vigorous pigs, yet no deaths occurred, nor were any ill effects of the transfer observed until this year. In view of this fact it is concluded that sow 1119, in addition to being in a poor physical condition as a result of lactation, had been further weakened by the invasion of the heart by *Strongyloides* larvae. As a result of this she was apparently unable to withstand the strain of unaccustomed exertion in the hot sun.

The observations herein reported confirm the previous findings that naturally acquired *Strongyloides* infections are capable of causing deaths of pigs and show, moreover, that under certain conditions these parasites may be pathogenic to sows. Sows may harbor an infection too light to be detected by ordinary methods of fecal examination, and yet this infection serves to infect their pigs and at the same time subjects themselves to repeated reinfections. Under these conditions the health of the sow may be injured to the extent that she will succumb to disease or to other conditions that would not materially affect a vigorous animal.

The value of phenothiazine for the removal of nodular worms from pregnant and nursing sows. John S. Andrews and J. W. Connelly, U. S. Bureau of Animal Industry.

In the course of investigations of swine parasites at the Coastal Plain Experiment Station, Tifton, Georgia, it was necessary to remove nodular worms from 15

TABLE 1.—*Efficacy of single 30 gram doses of phenothiazine for the removal of nodular worms from brood sows*

Sows treated		Date treated	Date of farrowing	Days between treatment and farrowing	Nodular worm eggs per gram of feces		Nodular worms passed following treatment		
Designation	Weight				Before treatment	After treatment	Males	Females	Total
Sows treated before farrowing									
	Pounds			*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
5 – 3	360	2/27/42	3/ 9/42	10	5,100	Very few	175	103	278
10 – 1[a]	345	do.	3/ 3/42	4	11,100	do.	640	306	946
19 – 1	315	do.	3/10/42	11	8,500	do.	204	282	486
10 – 1	345	9/ 1/42	9/ 8/42	7	Few	do.	34	47	81
12 – 4	335	do.	do.	7	do.	do.	621	528	1,149
16 – 10	330	do.	9/10/42	9	Many	do.	624	493	1,117
2 – 9	365	3/ 8/43	3/14/43	6	do.	0	566	812	1,378
5 – 4	365	do.	3/15/43	7	do.	4	102	228	330
16 – 4	320	do.	3/16/43	8	Many	0	27	40	67
4 – 5	350	8/21/43	8/27/43	6	420	0	75	158	233
15 – 1	340	do.	8/29/43	8	2,700	5	686	1,126	1,812
15 – 9	335	do.	9/ 1/43	10	No count	0	191	288	479
17 – 4	345	do.	8/29/43	8	do.	0	49	102	151
Sows treated after farrowing									
2 – 4	340	9/15/41	9/ 4/41	11	11,200	4	466	669	1,135
2 – 6	330	do.	9/ 8/41	7	1,800	1	105	309	414
14 – 3	305	do.	9/10/41	5	Many	1	63	200	263

[a] Treated on two separate occasions and appears twice in table.

brood sows very soon after they were put on the farrowing lot, only within a few days of the farrowing date. Phenothiazine was the anthelmintic chosen because it was known to have a high efficacy for the removal of nodular worms from pigs and could be administered in the feed. Accordingly, 30 grams of this compound, mixed with approximately 1 pound of tankage, were given to each animal without preliminary fasting; this is a dose rate of approximately 0.1 gram per pound of body weight.

In view of the fact that some investigators have reported that unsatisfactory results may follow the administration of phenothiazine to swine, the present account of the successful treatment of 12 sows from 4 to 11 days before farrowing and of 3 sows from 5 to 11 days after farrowing, is reported.

The data obtained from the sows before and after treatment with phenothiazine are given in the accompanying table.

The data in the table show that (1) in all but two instances where fecal examinations were made prior to treatment, nodular worm eggs were found in the feces of the sows, (2) from 67 to 1,812 nodular worms were recovered from the feces of the animals following treatment, and (3) in all cases, except sows 15–9 and 17–4 which were not examined prior to treatment, the number of nodular worm eggs per gram of feces passed after treatment was smaller than that found in feces prior to treatment. It appears, therefore, that the 30-gram doses of phenothiazine were effective in removing the vast majority of the nodular worms harbored by the animals prior to treatment.

No demonstrable ill effects were produced in any of the sows by the administration of phenothiazine, even though in one case only 4 days elapsed between treatment and farrowing. The pigs farrowed by the treated sows were not affected adversely by the treatment so far as could be determined by comparing their growth rates and general physical conditions with pigs of approximately the same age farrowed by untreated sows. In a few instances the phenothiazine appeared to cause constipation, but this condition may have been a result of the change in type of feed incident to the removal of the sows from pasture to a lot bare of vegetation where the test animals were temporarily confined during treatment.

CONCLUSIONS

The results of this work indicate that the administration of 30-gram doses of phenothiazine to the 15 brood sows successfully removed nodular worms from the animals without causing demonstrable harm to them or to the pigs farrowed within 4 to 11 days of the date of treatment.

Observations on controlling horse strongyles with repeated small doses of phenothiazine. A. O. Foster and R. T. Habermann, U. S. Bureau of Animal Industry.

In view of the demonstrated action of phenothiazine against horse strongyles (Harwood *et al.*, 1940, this journal 7(1): 18–20), 4 Chincoteague ponies and 1 Shetland pony were purchased in June, 1940, in order to test preliminarily the feasibility of employing this drug as a measure for controlling the parasites in question. Because contemporary work in the Zoological Division (Shorb and Habermann, 1940, Vet. Med., 35(8): 454–457), had already suggested that the daily consumption by sheep of as little as one-half gram of phenothiazine inhibited the maturation of infective stages of certain common nematode parasites in the feces of the treated animals, the decision was made to test a somewhat similar but more practical regime of medication on the ponies.

The observations were made at the Beltsville Research Center, Agricultural Research Administration, Beltsville, Md. The animals weighed from 275 to 345

pounds, were pastured on infested grounds, and medication consisted in giving 5 grams of phenothiazine weekly in feed to each animal. One (pony no. 2) was given this dose every Monday, another (pony no. 1) was given 2½ grams every Monday and Thursday, and two (ponies nos. 3 and 5) were given 1¼ grams on Mondays, 2½ grams on Thursdays, and 1¼ grams on Saturdays. Pony no. 4, a pregnant cross-bred mare, was left untreated, partly on account of her condition and partly to have a check on the hematocrit changes and infection levels of an untreated animal. Of the principals, 3 were given a total dose of 100 grams, the first treatments being given on July 29, 1940, and 1 (pony no. 5) was given a total

TABLE 1.—*Strongyle egg counts and hematocrit readings on 3 ponies to which 5 grams of phenothiazine were given weekly in feed. (E.P.G. = estimated eggs per gram of feces)*

Date, 1940	Pony No. 1		Pony No. 2		Pony No. 3	
	E.P.G.	Hematocrit	E.P.G.	Hematocrit	E.P.G.	Hematocrit
June 26	1334		267			
27	1334		267		.....	
28	1400		240		.....	
July 2	.....		.....		1400	
3	.....		.....		2800	
29	1400		400		1200	
29	Treatment begun					
August 5	1600		200		1200	
8	.....		.....		600	
12	90		40		140	
19	30		+		30	
26	224		3		2	
September 5	0	32	0	34	134	30
9	0	30	0	34	100	32
16	0	32	134	32	0	31
23	0	28	0	32	0	29
October 1	+	31	0	34	0	29
7	0	30	0	33	0	31
15	0	31	0	35	0	34
22	0	33	0	35	0	36
November 6	0	34	0	39	0	34
22	0	35	0	37	0	34
28	0	33	0	33	0	31
December 13	0	30	0	33	0	28
20	0	34	0	36	0	30

dose of 70 grams, the first treatment being given on September 9. The observations were terminated during the week of December 9 to 14. Meanwhile, the effect of the medication was followed by dilution or flotation counts of the number of strongyle and/or ascarid eggs passed in the feces. Shortly after the institution of treatment, it became evident from concurrent observations on other animals that large doses of phenothiazine, and frequently standard therapeutic doses, produced varying degrees of anemia in equines; hence hematocrit readings were made at more or less regular intervals during the second to fifth months of treatment.

The data on the 3 Chincoteague ponies are given in table 1. The Shetland pony exhibited an extremely low level of parasitism from the start, hence the data are of value only from the standpoint of toxicity, while the blood and feces of the control animal were examined relatively infrequently. From the data in the table, it will be noted that there were reductions in the egg productions of the 3 ponies as early as the second week of therapy and that there was no measurable acquisition of strongyle infection thereafter. The hematocrit readings suggest also that there

was no significant anemia caused by the drug. Moreover, 2 of the treated ponies that were mostly white-haired were watched for evidences of photosensitivity but none was noted. Throughout the time of these observations, the untreated animal showed egg productions of from 3000 to 4800 eggs per gram of feces, and the hematocrits ranged from 36 to 49.5 per cent of packed red cells. The readings on this animal were uniformly higher than those of the test animals but this difference is clearly coincidental and not associated with an absence of treatment. The hematocrit determinations on the Shetland pony showed extremes of 27.3 and 40.3, the latter level noted after 5 weeks of treatment. The average of 4 pretreatment readings was 31, of 11 determinations during the course of treatment, 34.

Ascaris infections were unaffected by this therapy. One animal, showing a few ascarid eggs when the tests were initiated, maintained its infection throughout the period of therapy and another acquired an infection during this period.

A brief summary of these findings was published in the Report of the Chief of the Bureau of Animal Industry, 1941, page 76. Since that time, other investigators have provided additional information on the advantages of giving divided doses of phenothiazine to horses, chiefly having in mind, however, the objective of avoiding toxic reactions. The present studies deal more directly with aspects of prevention and control, and the findings suggest that this approach should be pursued further.

Some instances of interspecific copulation among equine Strongylidae (Nematoda) and their probable significance. A. O. Foster, U. S. Bureau of Animal Industry.

During his studies upon equine strongylids, Looss (1902. Rec. Egypt. Govt. School Med., Cairo, 1901, p. 72) found a male of *Strongylus equinus in copula* with a female of *S. edentatus*. This occurrence was associated with an absence of females of the former species from the infestation which yielded the anomaly. Later (p. 117) in his remarks upon the cylicostomes, Looss stated that he examined 300 copulating pairs in regard to this point and found four instances in which the two sexes were of different species. He, at first, considered that these occurrences might effect the production of hybrid offspring and that herein was to be found an explanation of the "well-marked aberrations from the normal structure shown by a number of representatives of a species." In reality this view was prompted by his original confusion of *Cyathostomum labratum* and *C. labiatum*. In comment upon this Looss stated, "With the recognition of these two species, the last reason for admitting the occurrence of individual variations in the important and distinctive specific characters vanishes" (p. 91). The writer is advised that others have witnessed similar instances of interspecific copulation within this group of parasites, although it does not appear that their observations have been generally recorded. Lest it be thought that such occurrences are common, and without significance, it is desired to report the following observations upon this phenomenon.

During 3 years of study upon equine parasites, while the author was serving as Helminthologist to the Gorgas Memorial Laboratory, Panama, R. P., 6 instances of interspecific copulation were encountered in the course of routine determinations of species. They were as follows:

1. *Strongylus edentatus* male and *Cylicostephanus minutus* female.
2. *Strongylus vulgaris* male and *Cyathostomum coronatum* female.
3. *Cylicocercus catinatus* male and *Cylicocyclus nassatus* female.
4. *C. catinatus* male and *Cylicocercus pateratus* female.
5. *C. nassatus* male and *Cyathostomum labratum* female.
6. *C. nassatus* and *C. catinatus* males and *C. nassatus* female.

The above cases were met with in the course of individual determinations upon

well over 100,000 strongylid worms, representing 35 species. Data of another nature on over 85,000 of these were summarized in 1937 (Foster and Ortiz, Jour. Parasit., 23(4): 360–364). Although a careful account was kept of the physiological and morphological irregularities that were encountered, and the anomalous specimens preserved separately, it is unfortunate, in so far as the present records are concerned, that the total number of pairs *in copula* (after fixation and clearing) was not recorded. Yet it cannot but have been the experience of all who have studied equine Strongylidae that worms *in copula* represent a significant part of the average infestation. A high proportion of copulating pairs may be regarded as generally characteristic of the stouter bursate nematodes; it has been observed regularly by the author in infestations with *Ancylostoma*, *Kalicephalus*, and others. When it is considered that the average infestation with strongylid worms among the minimal infestations that were quantitatively studied by the author (*loc. cit.*) was about 1000 worms, representing on the average 15 different species, it is remarkable that the instances of interspecific copulation were so few. Indeed, the significant aspect to those of us who identified specimens was the almost incredible regularity with which worms were mated with members of their own species.

It is very doubtful, of course, that these "mismatings" ever produce hybrid offspring. The above-named species are constant morphologically and are among the commonest parasites of equines everywhere. Genotypical hybrids are perhaps a possibility, but this would seem to postulate the existence of some forms which, like the mule, are sterile. The occurrence, however, of phenotypical hybrids, differing structurally from the parents and presumably combining characters of two or more species, has probably never been observed.

Two important interpretations seem to be indicated by these observations, viz., that the several species are constant (except, of course, in the phylogenetic sense) and, corollary to this, that the strongylid parasites of equines are a standardized group of species. Much evidence in this direction has already been adduced. Further evidence is contained in the fact that, although descriptions of allegedly new species of cylicostomes appear frequently, probably all of the species proposed since Theiler's *Cylicostephanus asymetricus* in 1923 have been found to be synonymous with forms which, for the most part, are well known. The exceptions, apparently, are *Trichonema skrjabini* Erschow, 1930, and *Cylicostephanus parvibursatus* Vaz, 1931. The writer is unable to comment upon Erschow's species, but it is probable that Vaz's species will go into synonymy. The latter species was founded upon a single male specimen which is apparently not available for examination. In the absence of knowledge of the configuration of the posterior end of the mature female, it is doubtful if this specimen could have been assigned with certainty to the genus *Cylicostephanus*. Indeed, it has seemed from the measurements and figures presented by Vaz that he was dealing with *Cylicocercus goldi*. Hitherto undescribed species of cylicostomes will probably be found; but there is reason to emphasize that the group in question is relatively standardized and that those who study equine Strongylidae would avoid confusion by insisting that unusual specimens be allocated to known species unless there is satisfactory evidence that they cannot be.

Preliminary tests of perthiocyanic acid as a teniacide. Frank D. Enzie, U. S. Bureau of Animal Industry.

The testing of miscellaneous substances for anthelmintic properties revealed one, namely, perthiocyanic acid, which exhibited teniacidal action in dogs. Since available teniacides are not uniformly reliable in any host animal, a limited number of tests were conducted in order to ascertain the efficacy and safety of this chemical in dogs and, in addition, to determine to a limited extent whether or not it was of

promise as an anthelmintic in other host animals. Perthiocyanic acid is a light, orange-colored powder of fine texture, relatively insoluble in water. Chemically, it is a mixture of normal and iso-forms, having the empirical formula $H_2S_3C_2N_2$.

The tests were conducted at the station of the Zoological Division, Beltsville Research Center, Agricultural Research Administration, Beltsville, Maryland. The drug was administered in hard gelatin capsules after the test animals had eaten approximately one-half the regular feed. When a purgative was used, it was given from 2 to 3 hours after treatment, and consisted in magnesium sulfate given in capsules. The feces were examined daily for parasites in the usual manner. After 5 to 13 days the dogs were necropsied, and the intestinal tracts were examined for parasites. The data are given in the following protocols:

Dog No. 102; 12.0 pounds; 2.4 grams of the drug; vomited several hours after treatment; 5 *Dipylidium* strobilae with scolices and an undetermined number of strobilae without scolices recovered after treatment. At necropsy on fifth day no *Dipylidium* were found. Efficacy 100 per cent for *Dipylidium*.

Dog No. 103; 33.0 pounds; 6.6 grams of the drug; very poor condition; 17 *Taenia* strobilae with scolices recovered 4 hours after treatment; no toxic reactions 7 hours after treatment, but toxic reaction the next morning necessitated euthanasia. At necropsy no *Taenia* were found, and there was a marked congestion of all internal organs. Efficacy 100 per cent for *Taenia*.

Dog No. 107; 59.0 pounds; 8.85 grams of the drug, followed in 2 hours with 10 grams of magnesium sulfate; vomited 1½ hours after treatment; 5 *Taenia* strobilae with scolices, 1 *Dipylidium* strobila with scolex, and several *Dipylidium* strobilae without scolices recovered after treatment. At necropsy on seventh day no *Taenia* or *Dipylidium* were found. Efficacy 100 per cent for *Taenia* and *Dipylidium*.

Dog No. 108; 18.5 pounds; 2.78 grams of the drug, followed in 2 hours with 4 grams of magnesium sulfate; vomited after receiving magnesium sulfate; 4 *Dipylidium* strobilae without scolices recovered after treatment. At necropsy on tenth day recovered 2 immature *Dipylidium* 4.5 to 5.0 cm. Efficacy 50 per cent for *Dipylidium*.

Dog No. 109; 18.5 pounds; 1.85 grams of the drug, followed in 2 hours with 4 grams of magnesium sulfate; 15 *Taenia* strobilae, 13 with scolices, recovered after treatment. At necropsy, on thirteenth day, recovered 10 immature *Dipylidium* 3.0 to 9.0 cm. Efficacy 100 per cent for *Taenia* and 0 per cent for *Dipylidium*.

Dog No. 78; 46.0 pounds; 4.6 grams of the drug, followed in 2 hours with 8 grams of magnesium sulfate; vomited after receiving magnesium sulfate; 19 *Dipylidium* strobilae without scolices recovered after treatment. At necropsy on the eleventh day no *Dipylidium* were found. Efficacy 100 per cent for *Dipylidium*.

Dog No. 110; 44.0 pounds; 2.2 grams of the drug (recrystallized), followed in 3 hours with 5 grams of magnesium sulfate; 14 *Taenia* strobilae with scolices recovered after treatment. At necropsy on eighth day recovered 18 *Taenia* and 42 immature *Dipylidium* 1.0 to 4.5 cm. Efficacy 44 per cent for *Taenia* and 0 per cent for *Dipylidium*.

Dog No. 135; 29.0 pounds; 2.9 grams of the drug, followed in 3 hours with 4 grams of magnesium sulfate; vomited about 2 hours after treatment; 24 *Dipylidium* strobilae without scolices and 1 short *Dipylidium* strobila with scolex recovered after treatment. At necropsy on seventh day, no *Dipylidium* were found. Efficacy 100 per cent for *Dipylidium*.

Dog No. 136; 21.0 pounds; 2.1 grams of the drug, followed in 3 hours with 4 grams of magnesium sulfate; vomited 1½ hours after treatment; 3 *Dipylidium* strobilae without scolices recovered after treatment. At necropsy on seventh day no *Dipylidium* were found. Efficacy 100 per cent for *Dipylidium*.

Dog No. 147; 15.0 pounds; 1.5 grams of the drug, followed in 3 hours with 2 grams of magnesium sulfate; 8 *Dipylidium* strobilae, 3 with scolices, recovered after treatment. At necropsy on tenth day recovered 9 immature *Dipylidium* 1.5 to 9.0 cm. Efficacy 25 per cent for *Dipylidium*.

From the data presented in the protocols it is evident that perthiocyanic acid, administered at dose rates varying from 0.1 to 0.2 gram per pound of body weight, removed 100 per cent of 37 *Taenia* from 3 dogs and 73 per cent of 78 *Dipylidium* from 8 dogs. At a dose rate of 0.05 gram per pound, a dosage apparently below the therapeutic minimum, the recrystallized drug removed 44 per cent of 32 *Taenia* but none of 42 immature *Dipylidium* from 1 dog. The efficacies in all instances were based upon the effect of the drug on the scolices. Moreover, the immature *Dipylidium* recovered at necropsy 8 to 13 days after treatment were not considered as reinfections although it is reported that these tapeworms may reach sexual maturity in about 20 days, the mature worm attaining a length of approximately 15 to 40 cm. Consequently, it is believed that the calculations of efficacy were definitely conservative. The drug exhibited negligible anthelmintic action against ascarids (15/235), hookworms (0/836), and whipworms (0/362).

Since the toxic reactions exhibited by Dog No. 103 were not manifested until more than 3 hours after the anthelmintic action, the use of a purgative 2 to 3 hours after treatment seemed indicated. Each of 3 dogs was given the drug at a dose rate of 0.2 gram per pound and, after 2 to 3 hours, 2 of the dogs were given magnesium sulfate. One of the latter vomited shortly after receiving the drug and immediately ate the vomitus; the other dog vomited about 2 hours after receiving the magnesium sulfate and immediately ate a portion of the vomitus. There were no other untoward reactions in these dogs. The dog which was not given a purgative, however, exhibited inappetence, depression and blood-tinged feces for 2 days; this dog was apparently normal on the fourth day. One dog, weighing 33.0 pounds, was given 9.9 grams in the usual manner followed by a dose of magnesium sulfate after 5 hours. This dog had been given a large amount of raw beef the day before treatment, and the day after treatment large pieces of undigested beef were found in the feces. The only untoward reaction was considerable distress on defecation; the dog was apparently normal on the second day. This indicates, perhaps, that the drug might be less toxic if dogs are given easily digested feed for a day or two before treatment. In order to test the accumulative toxicity of the drug, one dog weighing 30.0 pounds was given 16 therapeutic doses over a period of 18 days, Sundays excluded. The first indication of toxicity appeared in the evening after the second dose and consisted of soft, blood-tinged feces. Subsequent observations disclosed rather marked toxic reactions; and at the end of the test period, the dog was submitted to necropsy. The most salient feature observed at necropsy was marked congestion throughout the carcass.

Gross and microscopic lesions were insignificant in all animals which received therapeutic doses of perthiocyanic acid, and they could not be attributed solely to the use of the drug.

When the drug was administered in capsules to 2 sheep at a dose rate of 0.1 gram per pound of body weight, it was toxic and without significant teniacidal action. Inappetence was induced when the drug was given to a tapeworm-free goat at a dose rate of 0.25 gram per pound of body weight. At 0.1 gram per pound of live weight, the drug produced slightly bloody feces in 1 of 2 cats; another cat was given 0.05 gram per pound of the recrystallized product without untoward results. None of the cats was infested with tapeworms.

Further investigation with perthiocyanic acid as a potential teniacide in dogs appears to be warranted. The most satisfactory results may be obtained when the drug is administered without a fasting period at a dose rate of 0.1 gram per

pound of body weight, with a maximum dosage of, perhaps, 5 grams. The use of a purgative such as magnesium sulfate 2 to 3 hours after treatment appears to be advantageous.

Normal agglutinins in vertebrate sera for *Trichomonas foetus* (Protozoa).[1]

Banner Bill Morgan, Department of Veterinary Science, University of Wisconsin.

The sera of many vertebrates normally contain agglutinins for trichomonads. This factor should be taken into consideration when devising a diagnostic test employing the agglutination reaction. The purpose of this paper is to present a list of animals with their corresponding normal agglutination titers to *Trichomonas foetus*.

Witte (1934) demonstrated normal agglutination in cattle sera, and weak agglutination with guinea pig sera. Twelve cows and 14 guinea pigs were tested. Endress (1939) described in great detail the agglutination reaction of *Trichomonas foetus* in normal cattle sera. Nelson (1937–1938), Endress (1939), Morisita (1939), Zeetti (1940), Robertson (1941), Schneider (1941), and Byrne (1942) showed that rabbit serum was weak in normal agglutinins. Endress (1939), Robertson (1941) and Schneider (1941) demonstrated strong agglutination titers in normal equine sera. Endress (1939) and Schneider (1941) tested chicken blood which contained very weak agglutinins for trichomonads. Endress (1939) also compared the sera of goat, sheep, and man. Kerr and Robertson (1941) reported on the use of the agglutination test for the diagnosis of trichomoniasis in cattle.

All sera used in the present investigation were heated at 56° C. for 20 minutes. Sera were never more than one week old when tested. The agglutination test was conducted in the following manner. The sera were diluted in small test tubes in the usual way with 0.7 per cent saline in a quantity of ½ cc. To this is added ½ cc of a suspension of a 48-hour pure culture of *Trichomonas foetus* in a concentration of 100,000 organisms per cc. The tubes were placed in a water bath at 37° C. for 2 to 4 hours. The trichomonads were cultivated in Schneider's (1942) citrate medium. The test suspension for the trichomonads was 0.7 per cent saline.

Trichomonas foetus used in this work was a strain isolated by Morgan and Wisnicky (1942) and subsequently studied by Morgan (1942). Care must be exercised in the choice of the trichomonad strain to be used for the antigen. Of 11 strains available in this laboratory, only 2 were suitable for this work. The strains which could not be used were too sensitive to saline controls. Further studies on the use of the agglutination and immobilization reaction for the diagnosis of bovine trichomoniasis has been reported in detail by Morgan (1943).

After incubation the trichomonads were pipetted on special Boerner (1940) slides with 12 mold-pressed raised rings. The readings were made with the low power of the microscope. Usually, 12 tubes were run starting with a dilution of 1:1 and ending with 1:1024. One 0.7 per cent saline control was employed.

The readings were marked as follows: ++++ = big round clumps with the flagella slightly motile, very few free organisms; +++ = big and medium clumps with a few free organisms; ++ = medium and small clumps with a large number of free organisms; + = small clumps or rosette formation with many free organisms; Tr = few rosettes with many free organisms; − = no clumps, no rosettes, all organisms free. Unless otherwise noted the results are based on an average of 10 different animals of each species. All animals used were considered full-grown adults.

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[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Project No. 622–V, Trichomoniasis and other reproductive diseases of cattle.

TABLE 1.—*Normal agglutinins in vertebrate sera for Trichomonas foetus*

Name of Animal	No. of animals	Dilution										
		1	2	4	8	16	32	64	128	256	512	1024
1. *Cyprinus carpio* (carp)	4	+	+	−	−	−	−	−	−	−	−	−
2. *Carassius auratus* (goldfish)	3	+	+	+	−	−	−	−	−	−	−	−
3. *Phrynosoma cornutum* (horned lizard)	8	+	+	−	−	−	−	−	−	−	−	−
4. *Rana pipiens* (leopard frog)	10	+	+	−	−	−	−	−	−	−	−	−
5. *Gallus g. domestica* (domestic chicken)	10	++	+	+	+	Tr	−	−	−	−	−	−
6. *Meleagris gallopavo* (domestic turkey)	10	+	+	+	+	+	−	−	−	−	−	−
7. *Numida meleagridis* (guinea fowl)	10	+	+	+	+	−	−	−	−	−	−	−
8 *Columba livia* (domestic pigeon)	10	+	+	+	−	−	−	−	−	−	−	−
9. *Streptopelia risoria* (ring dove)	6	+	+	+	−	−	−	−	−	−	−	−
10. *Macacus mulatta* (Rhesus monkey)	4	+++	++	++	++	+	−	−	−	−	−	−
11. *Homo sapiens* (human)	10	+++	+++	+++	++	+	Tr	−	−	−	−	−
12. *Rattus norvigicus* (common rat)	10	++	++	++	++	+	+	−	−	−	−	−
13. *Cavia coboya* (guinea pig)	10	++	++	++	++	++	+	−	−	−	−	−
14. *Lepus cuniculi* (domestic rabbit)	10	++	++	+	−	−	−	−	−	−	−	−
15. *Felis domestica* (domestic cat)	10	+++	+++	+++	++	+	Tr	−	−	−	−	−
16. *Canis familiaris* (domestic dog)	10	++	+++	++	++	+	+	+	+	Tr	−	−
17. *Vulpes fulva* (silver fox)	10	++	+++	++	++	+	+	+	−	−	−	−
18. *Mustela vison mink* (mink)	10	+	+++	++	+	+	−	−	−	−	−	−
19. *Equus caballus* (domestic horse)	10	++++	++++	++++	++++	++++	++++	+++	+++	++	+++	+++
20. *Sus scrofa domestica* (domestic pig)	10	++	+++	+++	++	++	+	Tr	−	−	−	−
21. *Ovis aries* (domestic sheep)	10	++	++	+	+	+	−	−	−	−	−	−
22. *Capra hircus* (domestic goat)	10	++	+++	++	+	+	+	−	−	−	−	−
23. *Bos taurus* (domestic cow)	10	++	++	++	++	++	+	+	+	−	−	−
24. *Odocoileus v. borealis* (deer)	6	++	+++	+++	++	++	+	−	−	−	−	−

A total of 24 species of vertebrates was tested, representing 5 classes, 11 orders, 19 families, and 24 genera (Pisces, Reptilia, Amphibia, Aves, and Mammalia), as shown in table 1. Carp, horned toad, and leopard frog sera were the weakest in demonstrable agglutinins with a titer of 1: 2. Among the birds, the dove and pigeon had a titer of 1: 4. In the mammalian group the rabbit was the weakest with a 1: 4 titer and the horse the strongest for normal agglutinins with a titer of 1: 1024.

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Physiological observations upon a larval *Eustrongylides*. VI. Transmission to various coldblooded intermediate hosts. Theodor von Brand, Department of Biology, The Catholic University of America, Washington, D. C.

It has been pointed out in a previous paper (von Brand and Cullinan, 1943) that larval *Eustrongylides* occur in a variety of fish species in the vicinity of Baltimore and Washington. J. B. Parker (personal communication) observed them in the eel, yellow perch, white perch, pickerel, catfish, large mouth black bass, crappie and rockfish, and the present writer found them also in bullfrogs obtained from the same region. The most frequently infected animals are however various species of *Fundulus*, especially *Fundulus heteroclitus.*

During the last three years several thousand infected *Fundulus* were dissected and all the worms found were alive, without a single exception. In the much smaller number of other hosts examined, on the other hand, many worms were dead and disintegrating. This latter observation is in line with the experience of Hunter (1937) who found many dead *Eustrongylides* in various fishes. This discrepancy

in behavior in different hosts is obviously open to several interpretations. It is possible that more than one species of *Eustrongylides* is involved. While it appears certain that the form parasitizing *Fundulus* is *Eustrongylides ignotus* (von Brand and Simpson, unpublished), no such definite statement can be made concerning the larvae observed in the other fish and the bullfrogs. Unfortunately a specific determination of these larvae is hardly possible. It may happen of course that different species have different life spans. An alternate explanation is that all the above named intermediate hosts harbor the same parasite species but that the worms have a different survival according to the organism invaded.

In view of this situation it appeared of interest to study whether larval *Eustrongylides ignotus* could be established in other intermediate hosts than *Fundulus*. Consequently larvae isolated from *Fundulus heteroclitus* were introduced orally, subcutaneously or rectally into a variety of coldblooded vertebrates as shown below. The experimental animals were then dissected after they had died or after having been sacrificed after varying periods.

1. *Fishes.*—The experiments with fishes as intermediate hosts are summarized in table 1. Crappies were used only for short time experiments since control fish caught at the same time and locality were found to be infected naturally (cysts especially in the muscles, but also along the mesenteries). There was no indication that the other fish used harbored natural infections.

The experiments as a whole obviously constitute proof that the larva of *Eustrongylides ignotus* can be established in other fish after having developed for an undetermined time in *Fundulus*. During the transfer a certain number of parasites died. The experiments on crappies indicate that this happens in the stomach. It can be suspected that the worms which were not recovered from the other fish species died also in the stomach. If they had perished in the tissues of the new intermediate hosts, remains of them would certainly have been found, at least in some cases.

It appears that those worms which succeed in leaving the alimentary tract of the new intermediate host crawl around in its body cavities or tissues for a certain time before settling down and becoming encysted. All the worms recovered alive appeared entirely normal. Three of the larvae encysted in the sunfish were used for a glycogen determination, using the micro-method described by von Brand (1936). The value obtained was 8.69 per cent glycogen of the fresh worm tissues. Worms taken from *Fundulus* at the time the larvae were fed to the sunfish yielded 8.41 per cent glycogen. Both values are obviously identical and show that the experimental worms were quite normal also in respect to their carbohydrate reserves.

The experiments summarized above represent therefore an indication that fish capable of capturing *Fundulus* may have acquired their infection in this way rather than by swallowing the as yet unidentified first intermediate host. Both ways of infection may of course exist simultaneously in certain fish. Why no worms were able to establish themselves in carp remains unclear. No case of natural infection of a carp has come to my attention. It may be significant that carp are vegetable feeders, while the other fish, and those found naturally infected, are predatory animals.

2. *Amphibia.*—Seven frogs (*Rana pipiens*) each received 2 and one frog received 3 larvae by oral feeding. The frogs were killed or died 3 to 17 days after infection, having been kept during this time at room temperature. The mortality of the frogs was high, 5 of them having died during the course of the experiment. It is rather questionable whether this is due to a detrimental action of the worms, since at the same time some uninfected control frogs were lost from unknown causes. Of the 17 worms used for these infections not a single one died or was lost. Seven were found in the muscles of various parts of the body, especially

TABLE 1.—*Fate of larvae of Eustrongylides ignotus introduced orally into various fishes after having been isolated from Fundulus*

Fish	Number of larvae introduced	Temp. of water, °C.	Duration of experimental period	Number of worms			Location of worms recovered	
				Recovered		Not accounted for	Alive	Dead
				Alive	Dead			
Crappie 1 (*Pomoxis sporoides*)	8	14	5 hours	8	...	.....	5 in stomach, 3 in small intestine	.....
Crappie 2	8	14–15	20 hours	5	3	.....	4 free in body cavity, 1 boring into muscles	Stomach
Crappie 3	8	14–15	39 hours	6	2	.....	3 free in body cavity, 2 boring into muscles, 1 in mesenteries	Stomach
Yellow Perch 1 (*Perca flavescens*)	5	11–14	7 days	5	...	.....	3 free in body cavity, 1 free in swim bladder, 1 encysted below peritoneum	.....
Yellow Perch 2	5	11–14	7 days	1	...	4	Muscles of body wall	.....
Yellow Perch 3	5	11–14	14 days	4	...	1	1 encysted in muscles, 3 burrowing in liver	.....
Catfish (*Ameiurus* sp.)	6	10–16	45 days	1	...	5	Encysted in mesenteries	.....
Sunfish (*Helioperca* ?)	14	10–17	30–46 days[a]	6	...	8	2 encysted in mesenteries, 2 encysted below peritoneum, 2 free in swim bladder	.....
Carps 1, 2, 3 (*Cyprinus carpio*)	5–12	10–17	42–44 days	...	...	5–12	.....	.....

[a] Worms had been fed to the fish over a period of 16 days.

those of the abdomen and thigh. In these cases little indication of cyst formation was seen. One worm was lying free in the dorsal lymph sac. Four were recovered from the ovary where in at least one case (14 days after infection) a distinct cyst had been formed. Three larvae had encysted in the mesenteries (13 and 17 days after infection). One worm was found lying partially free in the body cavity, its anterior end having penetrated the urinary bladder. The last worm was encountered in a similar position but its anterior end was buried in the kidney.

One *Necturus maculosus* was infected rectally with 2 larvae. It was killed after 21 days and 1 living worm was recovered. It was lying curled up, but not encysted in the body cavity.

Attempts to feed larvae orally to an *Amphiuma tridactylum* failed. The animal was always able to regurgitate them. Two worms were therefore implanted subcutaneously in the middle region of the body. After 42 days one living worm was recovered immediately under the skin at the site of implantation. The second worm was also alive and was found in an abscess-like cavity deep in the muscles of the body wall near the place of introduction.

The above experiments indicate therefore that various species of amphibia are potential intermediate hosts for the larvae of the worm in question.

3. *Reptilia.*—A small alligator (*Alligator mississippiensis*) was fed orally 5 larvae and killed 81 days thereafter. Two worms were found encysted under the peritoneum of the abdominal cavity, 1 encysted under the pleura and finally 1 in a cyst in the mesenteries. All worms were alive and used for a glycogen determination. The value obtained, 8.91 per cent, was high and again in the normal range.

Three turtles (*Chrysemys picta*) also were used. Turtle 1 received orally 5 worms and was killed 5 days after infection. Four worms were recovered. Two were partially free in the body cavity but had begun to bore into the lung. Two were found in the wall of the stomach. They were lying curled up in a cavity between the main muscle layers. Sections revealed that as yet no cyst had been formed. This is the only case where worms had chosen a place nearing the locality they inhabit in the definitive host (glands of the fore stomach of herons). Turtle 2 was killed 21 days after having received orally 6 worms. Only a single worm was found. It was alive and boring in the liver. Finally turtle 3 was killed 30 days after having been fed 3 worms. Two living worms were recovered; they were found in thin cysts of the peritoneum.

A Garter snake (*Thamnophis sirtalis*) was fed 4 larvae and killed after 42 days. No worms were found.

The above experiments demonstrate that the larvae of *Eustrongylides ignotus* are able to live for a long time in some reptiles.

SUMMARY

Larvae of *Eustrongylides ignotus* isolated from cysts of *Fundulus heteroclitus* were introduced orally, rectally or subcutaneously into a variety of fish, amphibians and reptiles. In most of these animals, with the exception of carp and garter snake, a number of worms, usually the majority of the introduced specimens, were able to leave the alimentary tract. They wandered around in the body of the new intermediate host, but in many cases were finally confined to cysts. All the worms that had penetrated beyond the alimentary tract were recovered alive. They appeared normal and two glycogen determinations yielded normal values. It appears therefore that all these host animals are suitable as intermediate hosts for *Eustrongylides ignotus*. It is consequently possible that an animal capable of capturing other larger vertebrates and found infected with the larvae in question may have contracted the infection in this way. The ingestion of the first intermediate host, which as yet has not been identified but which is likely to occur in the life cycle of this helminth, is therefore not necessary in these cases.

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Nacobbus dorsalis, nov. gen. nov. spec. (Nematoda: Tylenchidae) producing galls on the roots of alfileria, _Erodium cicutarium_ (L.) L'Hér. GERALD THORNE and M. W. ALLEN, U. S. Bureau of Plant Industry, Soils, and Agricultural Engineering, Salt Lake City, Utah.

Roots of alfileria (commonly known as "filaree"), *Erodium cicutarium* (L.) L'Hér., collected April 16, 1943, by the junior writer from a sand pit near the mouth of Caliente Canyon southeast of Bakersfield, Kern County, California, bore galls closely resembling those caused by the root-knot nematode, *Heterodera marioni* (Cornu, 1879) Goodey, 1932, but when given a further examination under the microscope it was found that the causal agent was an entirely different nematode. Specimens were submitted to the senior writer who determined that the species involved was related to a form known as *Pratylenchus aberrans* (Thorne, 1935) Filipjev, 1936. A portion of the collection was then forwarded to G. Steiner who immediately noted that the larvae and the male tail were similar, if not identical, to illustrations published by N. A. Cobb in 1918 (Estimating the Nema Population of Soil. U. S. Dept. Agr., Bureau of Plant Indus., Agr. Technology Cir. No. 1; figs. 17 & 20). These illustrations were drawn from what Cobb had supposed to be a male and a small, blunt-tailed larva of the sugar-beet nematode, *Heterodera schachtii* Schmidt, 1871.

It is interesting to note how the above chain of events has finally cleared up the problem of Cobb's illustrations. These had been drawn so carefully by W. E. Chambers, who was also an excellent observer, that it was difficult to believe that errors had been made. The drawings clearly showed the esophageal glands of the larva extending back along the dorsal side of the intestine in a somewhat tandem arrangement while those of *Heterodera schachtii* are in a ventral position. The male tail exhibited a small definite bursa unlike any observed on *Heterodera schachtii* found during extensive collecting over all the best growing areas of the United States. Cobb stated that the specimens came from Colorado but the exact location is unknown.

When the senior writer described the species now known as *Pratylenchus aberrans* the first plan was to make it the type species of a new genus but, unfortunately, this was not done. The additional data now at hand justifies the procedure and the following generic and specific diagnoses have been prepared. The genus is named in honor of the late N. A. Cobb, who was the first to make observations on this most interesting group.

Nacobbus, nov. gen.

Diagnosis.—Tylenchidae. Sexual dimorphism present with the adult female transformed into a swollen, more or less irregularly shaped body while the male remains a typical filiform, tylenchoid nema. Esophageal glands lying dorsad along the anterior end of the intestine. Plant parasites forming galls or swellings on the roots of the host.

Type species.—*Nacobbus dorsalis*, nov. spec.

Relationships.—The gross morphology of the female *Nacobbus* together with its gall-forming ability gives one, at first glance, the thought of a possible rela-

tionship with the root-knot nematode, *Heterodera marioni,* but except for these two superficial characteristics there is little similarity. The general form and appearance of the male and young female are much more indicative of a relationship to *Pratylenchus* but the sexual dimorphism and the dorsal location of the esophageal glands place it, at best, as a very distant relative. Searching through the Tylenchidae we find sexual dimorphism and dorsad esophageal glands combined in only one other species, *Tylenchus similis* Cobb, 1893.[1] However, the sexual dimorphism of *T. similis* occurs as a degeneration of the male spear and esophagus—a far different type of dimorphism than that exhibited by *Nacobbus.* Among the Neotylenchinae the genus *Deladenus* Thorne, 1941, possesses at least one enlarged esophageal gland located dorsal to the intestine while several members of the genus *Neotylenchus* Steiner, 1931, frequently have dorsal extensions of the esophageal glands overlapping the anterior end of the intestine. The submedian glands of *Hoplolaimus coronatus* are also located dorsally.

Relationship with any of these known groups must be very remote but from the evidence at hand it is probably safe to assume that *Nacobbus, Tylenchus similis,* and perhaps *Pratylenchus,* represent widely divergent products of a common ancestry. Global collecting and careful taxonomy may eventually fill in the great gaps between them but for the present the question of relationship remains a matter of pure speculation.

Nacobbus dorsalis, nov. spec.

Adult female.—Length 1.2 to 1.6 mm; width 0.5 to 0.8 mm. Body (Fig. 1, H), enlarged into an ovoid or spheroid form with short, projecting neck, and an enlongated posterior portion which usually protrudes from the tissues of the gall. Cuticle of neck and posterior extension marked by distinct striae but these are not visible over the distended portion of the body. Lip region and spear retaining much of their original shape but head and neck modified in shape and size. Tail and elongated posterior portion of body also variform and rarely so symmetrical as that figured. Esophageal median bulb greatly developed but the basal bulb with its elongated glands is obscured by the tissues of the body. Vulva transverse, very small compared to the size of the eggs which must pass through it. Vagina with thick muscular walls. Oviduct thinly walled, frequently containing eggs. The single ovary is convoluted about the interior of the body and from it develop approximately 1,000 to 1,500 eggs which have an average size of 44 by 93 μ (40–52 by 82–110 μ). Segmentation and at least one moult take place within the body of the female. Anus and rectum very obscure, invisible on some specimens. Surrounding the female there was a thin layer of tissue but it was not determined if this was of nemic or plant origin.

Larva.—The first stage larva can be observed only by opening an egg in which it has just been formed. At this time it is sharp-tailed and averages about 0.45 mm long by 26 μ wide. The cuticle is strongly striated and the lateral fields are marked by 4 bright lines which occupy about $\frac{1}{5}$ of the body width. The well-developed spear is about 25 μ long. At this stage the esophageal glands may still be contained in the basal bulb of the esophagus or beginning to extend themselves back over the intestine. Usually there is evidence of the beginning of a second moult as tissues shrink from the cuticle of the tail and begin to form the blunt, rounded tail of the second stage larva (Fig. 1, F).

The second stage larva is the one most commonly found in the eggs. At first it averages about 0.41 mm in length but develops rapidly until near 0.5 mm. The

[1] Synonym: *Rotylenchus similis* (Cobb, 1893) Filipjev, 1936. Cobb's original name is here retained since Filipjev's designation to *Rotylenchus* was an error.

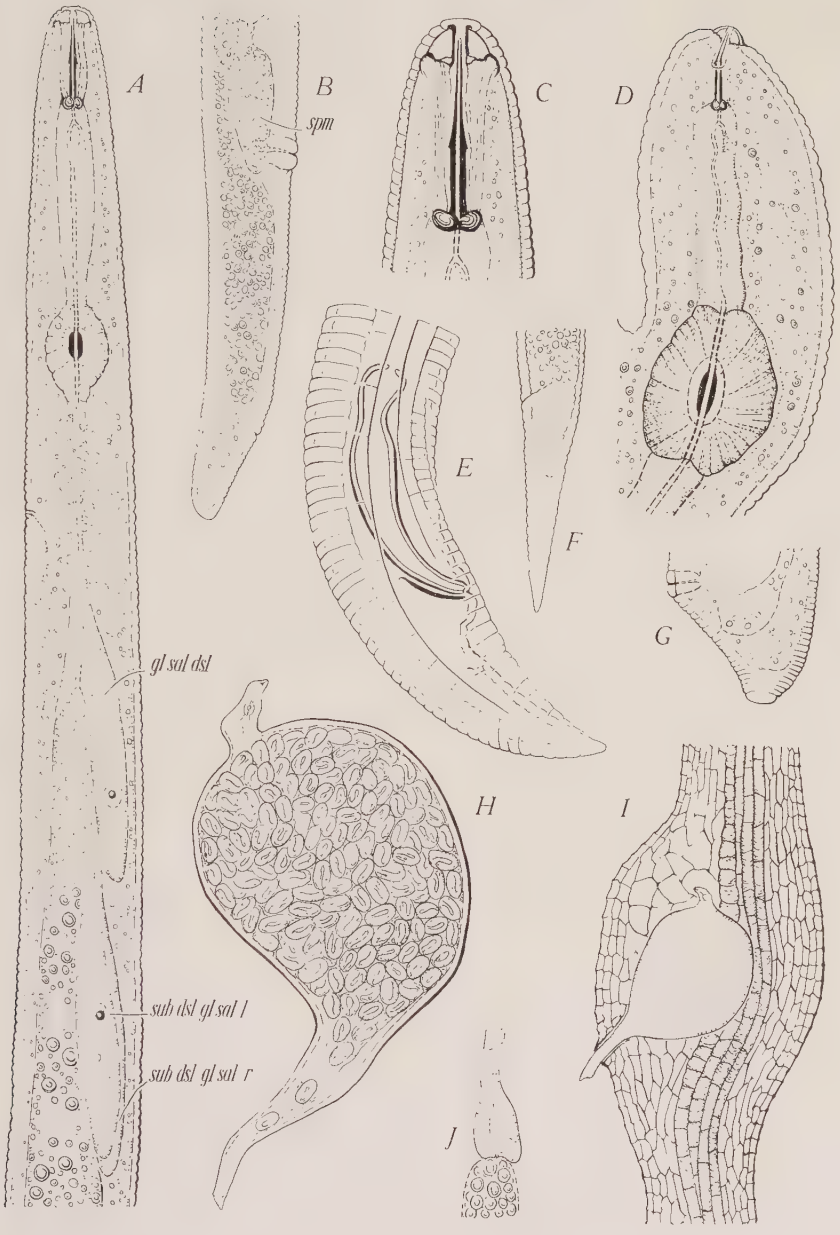

FIG. 1. *Nacobbus dorsalis.* A—Anterior portion of male showing arrangement of esophageal glands; *gl sal dsl,* dorsal salivary gland; *sub dsl gl sal l,* left subdorsal salivary gland; *sub dsl gl sal r,* right subdorsal salivary gland; ×600. B—Posterior portion of young female; *spm,* spermatozoa in uterus; ×600. C—Head of male; ×1200. D—Head of adult female; ×400. E—Posterior portion of male; ×1200. F—Posterior portion of first stage larva; ×600. G—Posterior portion of adult female; ×400. H—Adult female; ×50. I—Section through gall on root of *Erodium cicutarium;* ×20. J—Basal bulb of second stage larva from which the esophageal glands have not yet developed; ×600.

tail is bluntly rounded and very similar to that of the immature female (Fig. 1, B). Even at this stage an occasional specimen is found in which the esophageal glands have not yet formed into lobes (Fig. 1, J) but usually the glands are well developed and extend far back along the intestine.

Active or infective immature female.—Length 0.5 mm; $\alpha = 19$; $\beta = ?$; $\gamma = 22$; $V = {}^{30}82\%$. Stages between the larva and the immature female have not definitely been observed but there is probably one moult, and perhaps two. As will be noted, there is no appreciable increase in the average length over that of the second stage larva but the body is slightly more robust and the developing sexual organs have become a prominent feature of the anatomy. Most of these seen were fertilized and masses of spermatozoa were present in the oviduct and in the uterus at the entrance to the ovary (Fig. 1, B).

Adult male.—Length 0.8 mm; $\alpha = 28$; $\beta = 5.4$; $\gamma = 30$; $T = 55\%$. The deeply striated cuticle is marked on the lateral fields by 4 prominent lines or wings, the outer 2 generally being somewhat crenate. There is a tendency for the striae of the subcuticle to be minutely double. Four annules mark the lip region, the one surrounding the vestibule being more prominent. The massive spear and strongly developed esophagus are well illustrated and need no additional description (Fig. 1, A & C). The esophageal glands are a most interesting feature of the male for it is here that we can best observe them. Generally the arrangement is like that shown in figure 1, A, but occasionally the two submedian glands have a somewhat tandem arrangement. The dorsal gland always remains an integral part of the basal esophageal bulb. The intestine generally is packed with variable sized granules which largely obscure the cellular structure. Testis single, outstretched. Spicula large and strongly arcuate, resting upon a thin, trough-like gubernaculum. Bursa crenate, striated. Phasmids conspicuous with prominent connections leading in to the lateral cords but with only a minute fiber extending to the margin of the bursa. General cuticular pattern of tail as illustrated in figure 1, E. Some male tails were shorter than the one figured.

Diagnosis.—*Nacobbus* in which the female is ovate or spheroid with short neck and elongated posterior extension which usually protrudes from the gall in which it lives. Male tail $1\frac{1}{2}$ to 2 times as long as anal body diameter. Length of immature female tail equal to about one-third the vulva-anus distance. Eggs not deposited until at least one larval moult has occurred.

Type host.—Alfileria ("filaree"), *Erodium cicutarium* (L.) L'Hér.

Type locality.—California, U. S. A. (mouth of Caliente Canyon, southeast of Bakersfield).

Species of the genus *Nacobbus*

In addition to the type, one other species of *Nacobbus* is known and is here transferred to this genus:

Nacobbus aberrans, nov. comb. (Synonym: *Anguillulina aberrans* Thorne, 1935); *type host:* shadscale, *Atriplex confertifolia* (Torr. and Frem.) S. Wats.; *type locality:* Utah, U. S. A. (foothills west of Utah Lake). This species is readily distinguished from *N. dorsalis* by the variform females which do not possess an elongated posterior portion of the body protruding from the gall in which they live. The tail of the male is relatively shorter than that of the male of *N. dorsalis,* being about as long as the anal body-diameter. The tail of the immature, infective female is about one-half as long as the vulva-anus distance.

The identity of Cobb's material from Colorado cannot be determined from the two figures shown in his paper (*loc. cit.*). His figure 17 shows what is, without doubt, a male closely resembling that of *Nacobbus aberrans.* The larva illustrated by his figure 20 could have been a second stage form of either *N. aberrans* or *N.*

dorsalis. The species, therefore, must remain in doubt because of insufficient diagnostic characters.

PATHOLOGY

Detailed histological studies of gall formation have not been carried out and, therefore, a description of only the gross morphology can be presented.

The young female enters a small root and embeds its head near the vascular system. The opening made through the cortex remains and through it the posterior portion of the developing female protrudes, except in large galls where the distance is too great and in these the canal leading in toward the female remains open. Cells of the cortex and vascular system adjacent to the parasite become hypertrophied, resulting in the formation of a distinct swelling or gall in the affected portion of the root (Fig. 1, I). Certain endodermal cells immediately adjacent to the nema assume unusually large proportions and doubtless function much as the giant cells formed by the action of *Heterodera marioni*. Vascular tubes may be considerably hypertrophied but apparently continue to function, for root-growth appears to continue in an almost normal manner. Attack generally is made on the tap root but occasionally lateral roots bear galls also.

IMPORTANCE OF THE PARASITE

Visible injury to the host has not been observed and the parasite, therefore, is of no known economic importance. It is possible that a careful survey of plants in the vicinity where the collection was made will reveal other hosts, some of which may be of economic importance. During the summer of 1940, County Agent M. A. Lindsay sent 130 lots of root material from fields and adjacent foothills in Kern County to the Salt Lake Station of the Division of Nematology. When this material was examined occasional specimens of nemas were selected and preserved and among them is one male of *Nacobbus dorsalis*. Some of these lots were from the Caliente Canyon country but most of them were from cotton fields. The exact lot from which this male was secured is not known and it may have been from the canyon or from one of the fields.

''Filaree'' is frequently one of the most favorable plants to examine in making surveys for root-knot nematode in certain areas of the interior valleys of California and the finding of *Nacobbus dorsalis* immediately complicates the problem of identification, especially in the field. There is also the possibility of other plants' being attacked by it and, therefore, microscopic examination of all root galls will be necessary until a study has been made of its hosts and distribution.

Host-parasite relationships of the root-knot nematode, *Heterodera marioni*. I. The question of races. Jesse R. Christie and Florence E. Albin, U. S. Plant Industry Station, Beltsville, Md.

Some investigators have concluded that certain inconsistencies in the results of experiments or field observations on the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, are due to the existence of races of this parasite that differ from one another in their host–parasite relationships. For example, Sherbakoff (1939, Phytopathology **29**(8): 751–752) reported considerable root-knot injury to varieties of upland cotton grown on land previously planted to cotton but observed no injury to upland cotton grown on land previously planted to tomatoes even though the tomatoes had been severely injured by the disease. This investigator suggested, as a working hypothesis, ''that the host specialization of the nemas is due primarily to the segregation and survival of favored generic races.'' These observations by Sherbakoff and similar observations by others are convincing indications that races of this parasite exist and that they may be of considerable importance

in control procedures, especially those involving the use of resistant plants. Recent investigations by the writers corroborate this conclusion and demonstrate that differences in the host-parasite relationships of different races may become manifest in at least two ways: (1) a plant may be susceptible to one race and resistant to another; or (2) a plant may be susceptible to each of two races but the type of root galling produced by one race may differ from that produced by the other.

In the following discussion the term population is used to designate an aggregate of individuals of the root-knot nematode the progenitors of which were secured from a single plant or from several plants of the same kind that grew together in the same locality. The manner by which the parasites were secured and reared did not preclude the possibility that a population was composed of more than one race although in no instance did results seem to indicate that this was the case.

Recently undertaken investigation of root-knot-nematode races includes several experimental tests that involve a considerable number of different populations secured from various parts of the United States. The two tests reported in this paper were the first to be completed; they were exploratory in purpose and planned to test experimental procedures as well as to compare the behavior of different races. The tests were conducted independently, the first by Albin and the second by Christie. It is anticipated that the results of other experiments on this phase of the root-knot problem will be published later.

COMPARATIVE DEVELOPMENT OF DIFFERENT POPULATIONS IN PERSIAN CLOVER

Nine different populations of the root-knot nematode were used in this test the designations and sources of which are as follows:

Pop. 1	From	potato grown in region of Harlingen, Tex.
" 2	"	parsnip grown at Falls Church, Va.
" 3	"	sugarcane grown near Clewiston, Fla.
" 4	"	tropical yam, *Dioscorea* sp. grown near Brooksville, Fla.
" 5	"	"Pothos aureus," presumably *Scindapsus aureus*, grown near Orlando, Fla.
" 6	"	gladiolus corms grown near Meridian, Miss.
" 7	"	*Philodendron* sp. grown near Orlando, Fla.
" 8	"	*Hevea brasiliensis* grown near Posto Velho, Brazil.
" 9	"	potato grown near Klamath Falls, Oreg.

The test plant was Persian clover, *Trifolium resupinatum*. This plant proved especially suitable for the type of examination required because it grows slowly and, during a 30-day period, develops a root system of moderate size and of such a character that few roots are broken off when removed from the soil.

The inoculum consisted of root-knot-infected plant parts such as roots, corms, tubers, etc., cut into small pieces. The inoculum was carefully selected for uniformity but, no doubt, the number of viable eggs and larvae varied somewhat with different populations. In every instance, however, it was demonstrated by a microscopic examination that the inoculum contained an adequate number of viable eggs and larvae to bring about the infection of a susceptible plant.

For each population a 6-inch pot was filled with steam-sterilized soil to within about 1½ inches of the top. The inoculum was distributed evenly over the surface and covered with another half inch of soil. One hundred seeds of Persian clover were distributed over this surface and covered with one-fourth inch of sand. The plants were grown on a greenhouse bench and examined 30 days after the seeds were planted. The entire root system of each plant was examined critically under a dissection microscope and, so far as possible, every root-knot nematode was dissected out of the root tissues. Results of the examinations are shown in table 1. Only parasites whose condition was unquestionable are listed as dead.

The roots of the plants grown in soil infested with population 8 showed no evidence of having been invaded. If larvae entered the roots, which seems probable, in view of Barrons' results (1939, Jour. Agr. Research 28(4): 263–271), they died very promptly and without stimulating persistent abnormalities in root growth. Incidentally, when an attempt was made to infect tobacco plants with this population, using identical inoculum, an occasional partly-grown parasite could be found after about 3 months but no evidence of an infection after about 6 months. The roots of plants grown in soil infested with population 5 showed slight swellings or irregularities giving them a lumpy appearance. Without much doubt these roots had been invaded but the larvae had been unable to survive and, at the time of examination, no recognizable remnant of a parasite could be found. Plants infected with population 4 harbored a total of 56 parasites of which all except 10

TABLE 1.—*Comparative development of different populations of the root-knot nematode in Persian clover*

Population number	1	2	3	4	5	6	7	8	9
Date seeds were planted (1943)	2/20	3/2	3/4	3/31	4/8	4/15	5/25	5/27	9/3
Total number of plants secured	37	21	19	46	39	37	28	29	63
Total number of parasites	1005	707	84	56	0	139	569	0	214
Average per plant	27	33.8	4.5	1.2	0	3.8	20	0	3.4
Undeveloped larvae,									
Living	115	17	0	1	0	0	7	0	59
Dead	0	0	0	13	0	0	0	0	49
Partly developed parasites,									
Living	578	239	49	9	0	37	115	0	50
Dead	42	8	10	33	0	0	0	0	56
Metamorphosing males	4	60	0	0	0	0	0	0	0
Adult males	4	150	0	0	0	0	6	0	0
Full-grown females	225	233	25	0	0	102	441	0	0
Egg masses	84	175	43	0	0	42	267	0	0
Jelly-like deposits not containing eggs	0	0	0	0	0	53	180	0	0

were dead. Of those alive none had reached the molting stage. Plants infected with population 9 harbored a total of 214 parasites of which nearly half were dead. Of those alive none had become fully grown, over half having undergone little or no development. Plants infected with population 6 harbored a total of 139 parasites of which 102 were fully grown and 95 had reached the egg-laying stage. In spite of this relatively high proportion of fully grown females, the number of eggs per egg mass ranged from 2 to 27 with an average of slightly over 13, a much smaller number than in any other case where eggs had been deposited. Furthermore, the females had an abnormal appearance giving the impression that they were ill-nourished and in many instances the posterior end of the body protruded from the root. It seems probable that total egg output would have been very low. Of the 707 parasites harbored by plants infected with population 2, 210 were males. The present data do not seem to suggest that this sporadic abundance of males is correlated either with crowding or with an unsuitable host.

The results of this test indicate that Persian clover is not an equally suitable host for all the populations tested. This plant appears to be a suitable host for populations 1, 2, 3, and 7, a questionable host for 6, and an unsuitable host for 4, 5, 8, and 9. If Persian clover was not, in all cases, an equally suitable host, the populations must have included different races that differed from one another in their host-parasite relationships.

COMPARATIVE SUSCEPTIBILITY OF CERTAIN PLANTS TO DIFFERENT POPULATIONS

Fourteen populations of the root-knot nematode were used in this test, four of which were used in the preceding test. The designations and sources of these populations are as follows, population numbers in parentheses being the numbers used in the preceding test:

Pop. 1		From alfalfa grown near Shafter, Calif.
" 2		" cotton grown near Shafter, Calif.
" 3A		" potato grown near Reno, Nev.
" 3B (1)		" " grown in region of Harlingen, Tex.
" 3C		" " grown near Tampa, Fla.
" 3D		" " grown on Long Island, N. Y.
" 3E (9)		" " grown near Klamath Falls, Oreg.
" 3F		" " grown near Knoxville, Tenn.
" 4		" peanut grown in Pitt County, N. C.
" 5	(2)	" parsnip grown at Falls Church, Va.
" 6		" sweetpotato grown at Beltsville, Md.
" 7		" castilla rubber tree grown in greenhouse, Beltsville, Md. (propagated in a Florida nursery).
" 8		" tomato grown in greenhouse, Beltsville, Md.
" 9	(3)	" sugarcane grown near Clewiston, Fla.

The test was conducted in a greenhouse and 8-inch pots were used. For convenience, all pots inoculated with the same population are referred to as a series. The inoculum consisted of soil from pots in which root-knot-infected plants had been grown, in most cases tobacco, these plants having served to build up and maintain the various populations. The inoculum for each series was thoroughly mixed, approximately an equal amount apportioned to each pot, and sufficient steam-sterilized soil added to fill the pot. Hence, the potency of the inoculum was about the same for all pots of a given series but, no doubt, varied somewhat for different series. Two pots were provided in each series for each kind or variety of plant and from 1 to 3 plants were grown in each pot depending on the character of the plant. Ten different plants were tested, the names of which are given in table 2.

All plants were examined after a period of 8 weeks $\pm$ 1 day. The root system of a test plant was washed free of soil, submerged in water in a photographic tray, and examined under favorable light with the naked eye and with a hand lens. When a plant was moderately or heavily infected no further examination was made. In all other cases a root sample, clipped from various parts of the root system, was cleared and stained for more critical study. If roots were seen with abnormalities of questionable cause but suggesting root knot, these were included in the sample. The technic employed was the one described by McBeth, Taylor, and Smith (1941, Proc. Helminth. Soc. Wash. 8(1): 26) except that Sudan III was used as a stain instead of acid fuchsin or cotton blue and the roots were not washed in water after staining. Results are shown in table 2 where degree of infection is indicated by the following symbols:

0 No evidence of root knot seen.

1 An occasional parasite found by carefully searching cleared and stained roots; parasites immature, few having reached the molting stage; eggs not seen.

2 Parasites usually very scarce, sometimes moderately numerous but with full-grown females very scarce; eggs present but exceedingly few and found only by carefully searching cleared and stained roots.

3 Infection light. Parasites usually few, when moderately numerous mostly immature; an occasional full-grown female and an occasional egg mass here and there that could be seen on living roots without much searching.

4 Infection moderate. Full-grown females and egg masses moderately numerous.

5 Infection heavy. Full-grown females and egg masses numerous.

1 and 2 represent degrees of infection comparable to that sometimes designated a "trace" and in either case the infection would be difficult to detect in living roots, the most important difference between them being the presence or absence of eggs. 3, 4, and 5 represent degrees of infection comparable to those usually designated light, medium, and heavy, respectively.

TABLE 2.—*Comparative susceptibility of certain plants to different populations of the root-knot nematode*

Plant	Populations													
	1	2	3A	3B	3C	3D	3E	3F	4	5	6	7	8	9
Arachis hypogaea, peanut, variety Virginia Runner	0[a]	0	0	0	0	4	...	...	5	4	0	0	0	0
Fagopyrum sagittatum, buckwheat, variety Japanese	4	5	0	5	5	5	0	4	4	...	...	4	...	5
F. tataricum, buckwheat	4	3	0	...	...	...	...	...	4	...	...	...	...	0
Gossypium hirsutum, cotton, variety Coker 100	1	5	1	1	3	...	2	1	1	0	2	1	1	1
Lycopersicon, tomato, variety Rutgers	5	5	3	5	5	5	3	5	5	5	5	5	5	5
Medicago sativa, alfalfa, variety not known	5	2	0	...	1	4	...	5	4	4	0	3	0	5
Soja max, soybean, variety Laredo	3	2	0	0	1	2	...	1	3	...	...	3	...	2
Stizolobium, velvetbean, variety Florida	3	3	0	1	0	0	...	2	0	...	...	1	..	4
Vigna sinensis, cowpea, variety Iron	5	4	0	3	4	3	...	1	4	...	...	3	...	5
V. sinensis, cowpea, variety, Crowder	5	3	3	3	3	3	...	...	4	...	...	2	...	5

[a] See text for explanation of symbols.

The most striking differences in comparative susceptibility to the different populations were shown by peanut, cotton, and alfalfa. Peanut was quite highly susceptible to populations 3D, 4, and 5 but highly resistant to all the others against which it was tested. Cotton was highly susceptible to population 2, slightly susceptible to 3C and at least moderately resistant to all the others against which it was tested. Alfalfa was susceptible to populations 1, 3D, 3F, 4, 5, 7, and 9 but resistant to 2, 3A, 3C, 6, and 8.

While tomato was susceptible to all the populations the type of galling produced by some of them differed markedly from that produced by others. When infected with populations 3A and 3E the galls were small, inconspicuous, and mostly confined to the small roots, large roots near the base of the stem not being appreciably affected. When this plant was infected with populations 3D, 4, and 5 the galling was similar to that produced by 3A and 3E except that most of the galls developed numerous radiating rootlets which, in turn, bore galls with radiating rootlets, thus resulting in a reticulate root growth. Such plants frequently had a denser root system than normal ones. When this plant was infected with population 3C all roots, including large ones near the base of the stem, were greatly

swollen, many of the galls were large, and there was a conspicuous lack of fine roots. Populations 1A, 2, 3B, 3F, 6, 7, 8, and 9 produced galling similar to, though perhaps not identical with, that produced by 3C. Comparable differences in type of galling have been noticed with other plants but were most pronounced with tomato.

The results of this test indicate that these 14 populations of the root-knot nematode include at least 5 distinct races and probably more. It seems probable that populations 3A and 3E constitute one race, 3D, 4, and 5 constitute a second race, 2 a third race, and 1 a fourth. Population 9 may be identical with population 1. Populations 3B, 3C, 3F, 6, 7, and 8 probably do not constitute a single race but all appear to differ from the 4 races already mentioned.

Methods for differentiating and identifying different races remain to be developed. The possibility that some races may possess distinguishing morphological characters cannot, as yet, be ruled out. In the absence of such characters it will be necessary, presumably, to test a population against a series of differentiating hosts. Plants suitable for this purpose can be found only by trial. Type of galling would be the ideal criterion but it seems likely that this will serve for only a few races.

SIGNIFICANCE OF RACES IN ROOT-KNOT CONTROL

In many instances, especially where large acreage was involved, the use of resistant plants, in one way or another, has been the only practical method for controlling root knot. Recent developments in the use of soil fumigants, especially the introduction of cheaper materials, indicate that chemical treatment of the soil may be employed in the future on a much larger scale than has hitherto been practical. Even though this prospect materializes, the need for information regarding the comparative susceptibility or resistance of different plants to root knot will be by no means eliminated.

While recognition of the fact that there are distinct races of the root-knot nematode introduces new complications, also it introduces new possibilities and, what is equally important, explains many apparent inconsistencies in the results of experiments and field observations for which hitherto there seemed to be no logical reason. Frequently crops regarded as highly resistant to root-knot and recommended for rotations in controlling this disease have failed, in some instances, to show the expected resistance. Thereafter the crop either was not recommended for this purpose or was recommended reluctantly and with misgivings.

Peanuts have been considered a highly resistant crop and have been recommended for growing on root-knot infested land especially in the southeastern states. Peanuts are adapted to that region, can be grown profitably there, and can be used with satisfactory results in a rotation that includes tobacco. Occasionally, however, peanuts become rather heavily infected and recently reports of such instances have become increasingly common. The question arises, can this crop be recommended safely in a rotation for the control of root knot and if so under what circumstances? Results from the second test reported herein and from other observations on peanuts seem to justify certain conclusions that are pertinent to this question.

Peanut, variety Virginia Runner, was susceptible to only one of the races of root knot against which it was tested and highly resistant to all the others. This is not the race that causes "big root" on tobacco. While tobacco is susceptible to this race that infected peanut, injury is not severe; galls, sometimes numerous, are usually small and inconspicuous, large roots near the base of the stem are not appreciably affected, and the number of fine roots is not materially reduced. Even plants with a heavy infection might pass unnoticed unless examined critically. Incidentally, this race is easily distinguished from all the others thus far tested by the type of galling it produces on some plants including tobacco. Hence, if the

purpose of a rotation is to control root knot on tobacco there is reason to believe that peanuts can be recommended and should prove satisfactory. On the other hand, if the purpose of a rotation is to control root knot on potatoes or on parsnips and similar root crops the inclusion of peanuts is open to question. Potatoes, parsnips, and carrots are severely affected by the race to which peanuts are susceptible.

Admittedly, the above statements are based on tentative conclusions that need verification through additional experiments of greater scope. The matter is presented to illustrate how recognition of the fact that there are distinct races of the root-knot nematode may aid in formulating more intelligent control procedures when information is available regarding the identity and behavior of these races.

The correct authorities and dates for various supergeneric names in the nematode suborder Strongylina. ELLSWORTH C. DOUGHERTY, Department of Zoology, University of California, Berkeley, California.

Various names of groups within the suborder Strongylina—of the order Rhabditida, class Phasmidea, and phylum Nematoda—are habitually or generally associated in the current literature with incorrect dates or authorities. In some cases these errors are also to be found in the great compilation of supergeneric, generic, and specific names of "roundworms" (Nematoda, Nematomorpha, and Acanthocephala) by Stiles and Hassall (1920). Even in the case of the subordinal name itself there can be a difference of opinion as to whose authority it should be referred. These names are enumerated with brief comment in the following paragraphs.

1. Strongylina Pearse, 1936. The strongyline nematodes were first assigned subordinal status by Railliet and Henry (1913, p. 452). The paper in which this was done was not located by Stiles and Hassall as their index catalogue has the following entry: "Strongylata Rail. & Henry, 1913 [nv.]. . . ." As the earliest known authority they listed Skrjabin (1916). To my knowledge there has been hitherto no published statement as to the origin of Railliet and Henry's name except in a work by Neveu-Lemaire (1918, p. 7).

Chitwood (1937) in an appendix to a comprehensive paper on the classification of the phylum Nematoda, which at that time he treated as a class Nematoda, adopted within this phylum the uniform terminations for phyletic groups proposed by Pearse (1936). Although few helminthologists have followed this departure, I strongly subscribe to the idea behind Pearse's system and accordingly prefer to render the subordinal name as Strongylina instead of Strongylata. A problem thus arises as to how to accredit the name "Strongylina." Chitwood and Chitwood (1937) have adopted the policy of placing in parentheses after such group names the names of respective authors who first used the stems involved. They thus rendered the name under consideration as "Strongylina (Railliet and Henry, 1913)." This is a logical move. However, there is no generally accepted rule for such use of parentheses, and in fact supergeneric names have most generally been attributed to the first authority or authorities to spell them in the form now regarded as correct. For example, family names are attributed to the first person or persons to use the correct stem plus *-idae,* even though this same stem was used previously with a different ending for the same, or a similar group of familial rank. Following this custom the name under consideration would be rendered Strongylina Pearse, 1936. It will be argued on justified grounds that Pearse acted only as a compilator and unifier and that, as such, he does not deserve recognition as authority for the numerous group names which appeared with new terminations in his work of 1936. For this reason I accept his authority of "Strongylina" only in recognition of current practice which I do not wish to contradict without official backing. But

I do make the hearty recommendation that the General Council on Zoological Nomenclature, recently established in this country, take steps toward formulating a more definite treatment of supergeneric names, now largely ignored in the International Rules of Zoological Nomenclature.

2. Strongylinae Railliet, 1885. This subfamilial name is most generally given as Strongylinae Railliet, 1893. Stiles and Hassall listed it as Strongylinae Stossich, 1898, despite the fact that they recorded Railliet's prior usage (1893). However, it has apparently been generally overlooked that the name Strongylinae was actually first used by Railliet even earlier (1885, p. 330) and that it should therefore be designated Strongylinae Railliet, 1885. Accompanying this name were the first usages of Sclerostominae and Eustrongylinae, incorrectly given by Stiles and Hassall as Sclerostominae Railliet, 1898, and Eustrongylinae Stossich, 1899. However, neither name has valid status now inasmuch as the type genera of both have been eliminated as synonyms.

3. Cyathostominae Nicoll, 1927. The genus *Cyathostomum* was proposed by Molin (1861), but rejected by Looss (1902) because of the existence of a separate genus *Cyathostoma* Blanchard, 1849. Looss proposed the generic name *Cylichnostomum,* but it was later recognized by Railliet (1916) that *Trichonema* Cobbold, 1874, was earlier. However, a recommendation under Article 36 of the International Code expressly states that "when once introduced . . . [generic names differing by slight variations in spelling from others already in use] . . . are not to be rejected . . . ," and therefore there is no reason for not reverting to the generic name *Cyathostomum.* Since the subfamily Trichoneminae Railliet, 1916 (or correctly Trichonematinae Nicoll, 1927) has been recognized as a subfamily in the family Strongylidae Baird, 1853, Nicoll's substitution (1927) of Cyathostominae must be accepted. This has already been done by Chitwood (1937), but he has not been generally followed.

4, 5. Ancylostomatidae Nicoll, 1927; Ancylostomatinae Nicoll, 1927. Nicoll (1927) emended the family name Ancylostomidae Leiper, 1911[1], and subfamily name Ancylostominae Stephens, 1916, to Ancylostomatidae and Ancylostomatinae respectively because the stem of the Greek word *stoma* is *stomat-*, not *stom-*. For the same reason that I have accepted Strongylina Pearse, 1936, instead of Strongylina (Railliet and Henry, 1913) Pearse, 1936, I believe that with existing precedents the authorship of these names in their emended form should be credited to Nicoll alone.

6. Uncinariinae Rosenau, 1914. This subfamily of the family Ancylostomatidae is generally called Necatorinae Lane, 1917. An earlier name, Bunostominae Looss, 1911, is unavailable, as Cameron (1923) showed that *Bunostomum* Railliet, 1902, is a synonym of *Monodontus* Molin, 1861. Chitwood (1937) has accepted Uncinariinae as the correct name, but has not indicated its authority. It appears to have been first used by Rosenau (1914, p. 116) before Lane (1917) proposed the name Necatorinae. It should therefore be rendered Uncinariinae Rosenau, 1914.

7, 8. Metastrongylidae Leiper, 1909; Trichostrongylinae Leiper, 1909. These two names were established by Leiper in a paper which appeared in the *Third Report of the Wellcome Research Laboratories at the Gordon Memorial College, Khartoum,* the title page of this volume bearing the date 1908. It would appear, however, that the date of publication should be taken as February 2nd, 1909. This decision cannot be supported by the International Code, which is inadequate on the point of what constitutes publication for nomenclatorial purposes, but at the suggestion of Dr. W. H. Osgood (*in litteris*), Secretary of the General Council on Zoological Nomenclature, I turn to the code of the American Ornithologists' Union,

[1] This was first used by Leiper (1911), not by Lane (1917) to whom it is usually accredited.

which is specific in regard to this matter. Canon XLV reads: "Publication consists in the public sale or distribution of printed matter. . . ." That the date 1908 has come to be questioned at all has resulted from the fact that Leiper in the same paper also proposed as new the subfamily Metastrongylinae. But Railliet and Henry (January 22nd, 1909) had already erected a subfamily of this same name, in ignorance of Leiper's intentions. Railliet later (1916) pointed out the true date of publication of Leiper's work and claimed priority on the name Metastrongylinae for himself and Henry. However, he has been almost entirely ignored. I do not know how Railliet determined the correct publication date of Leiper's work, but I presume that he adopted a procedure similar to mine. I owe my decision to the fact that Messrs. Baillière, Tindall and Cox, publishers of the *Reports* of the Gordon Memorial College, and Dr. C. M. Wenyon, Director-in-Chief of the Wellcome Research Institution, have very kindly supplied me (*in litteris*) with the necessary information to satisfy me that February 2nd, 1909, was the first day of "public . . . distribution" of Leiper's paper in the sense of the Code of the American Ornithologists' Union. It may be mentioned that in this same paper the following nematode species were described as new: *Filaria bufonis, Physaloptera quadrovaria,* and *Heterakis numidae;* also the two trematode species *Gastrohylax wenyoni* and *Balfouria monogama,* with the new genus *Balfouria.*

9. Metastrongylinae Railliet and Henry, 1909. For reasons already given under nos. 7 and 8, Railliet and Henry's usage (Jan. 1909) of this name is considered to take prior status over Leiper's (Feb. 1909).

SUMMARY

By way of summarizing the information presented in the foregoing paragraphs I repeat here the classification into supergeneric groups given for the suborder Strongylina by Chitwood (1937) and added to by Chitwood and Chitwood (1937). I have in contrast to these authors included the authorities and dates for families and subfamilies. Where I have made comments, corrections, or recommendations, they are indicated by an asterisk [*] in front of each group name effected. Presentation of this classificatory scheme is not meant to indicate that I subscribe to it in all of its details; in fact I have previously (Dougherty, 1943) indicated that I believe that the family Pseudaliide Railliet, 1916, should not include the subfamily Filaroidinae Skrjabin, 1933, but should be restricted to the lungworms of porpoises.

* Suborder Strongylina Pearse, 1936
  Superfamily Strongyloidea Weinland, 1858
    Family Strongylidae Baird, 1853
      * Subfamily Strongylinae Railliet, 1885
      * Subfamily Cyathostominae Nicoll, 1927
      Subfamily Oesophagostominae Railliet, 1915
    Family Syngamidae Leiper, 1912
      Subfamily Syngaminae Baylis and Daubney, 1926
      Subfamily Deletrocephalinae Railliet, 1916
      Subfamily Stephanurinae Railliet, Henry, and Bauche, 1919
    * Family Ancylostomatidae Nicoll, 1927
      * Subfamily Ancylostomatinae Nicoll, 1927
      * Subfamily Uncinariinae Rosenau, 1914
    Family Cloacinidae Travassos, 1920
    Family Diaphanocephalidae Travassos, 1920
  Superfamily Trichostrongyloidea Cram, 1927
    Family Trichostrongylidae Leiper, 1912
      * Subfamily Trichostrongylinae Leiper, 1909
      Subfamily Ollulaninae Hall, 1916
    Family Heligmosomidae Cram, 1927
  Superfamily Metastrongyloidea Lane, 1917
    * Family Metastrongylidae Leiper, 1909
      * Subfamily Metastrongylinae Railliet and Henry, 1909

Family Pseudaliidae Railliet, 1916
Subfamily Pseudaliinae Railliet and Henry, 1907
Subfamily Filaroidinae Skrîabin, 1933

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Report of the Brayton H. Ransom Memorial Trust Fund

December 31, 1942

On January 21, 1942, the trustees voted an award of $25.00 for 1941, to the Proceedings of the Helminthological Society of Washington.

On July 12, the holder of the $1,350.00 loan repayed that amount to the Trust Fund.

On October 27, $1,400.00 from the Fund was invested as a loan at 4 per cent interest, the note being covered by a life insurance policy as collateral, with interest payable semiannually.

The status of the Fund, since the previous report in the Proceedings of the Helminthological Society, January, 1942, is as follows:

On Loan, January 1, 1942		$1350.00
Balance on Hand, Jan. 1, 1942		119.24
Receipts:		
Interest on loan to Jan. 12		27.00
Bank interest to June 30		1.20
Interest on loan to July 12		27.00
Repayment of loan, July 12		1350.00
Bank interest to Dec. 31		4.82
Total Receipts		$1529.26
Disbursements:		
1942 rent, safe deposit box	$3.50	
tax	.70	4.20
Award to Helminthol. Soc. Proc.		25.00
As loan at 4 per cent interest		1400.00
Total Disbursements		$1429.20
Balance on Hand, December 31, 1943		100.06
		$1529.26

Eloise B. Cram,
Secretary-Treasurer

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